ANNALS
OF THE
ENTOMOLOGICAL SOCIETY OF AMERICA

Volume 53 July, 1960 Number 4

ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

Published by the

ENTOMOLOGICAL SOCIETY OF AMERICA

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A bimonthly journal published on the 15th of January, March, May, July, September, November, devoted to the interest of classical entomology. Authors are entirely responsible for statements in the *Annals*, whether of fact or opinion.

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Second class postage paid at Columbus, Ohio. By order of Dec. 10, 1958.

Printed by The Spahr & Glenn Company,
50 East Broad Street
Columbus 15, Ohio, U. S. A.

Issued July 15, 1960

Annals of
The Entomological Society of America

Volume 53 JULY, 1960 No. 4

OBSERVATIONS ON TICKS (IXODOIDEA) OF LIBYA[1]

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ABSTRACT

Details are provided concerning 14 species of ticks of which the authors have seen Libyan material. Insufficient search for ticks in this area probably is responsible for lack of records of about a dozen other species, which are discussed here. The status of five others, reported from Libya in the literature, is considered uncertain, published records for three additional species appear to be incorrect, and another one involves a nomen nudum. The importance of certain small wild animals as hosts of *Hyalomma* spp. in immature stages is noted. *Hyalomma marginatum turanicum* and *H. m. rufipes* probably were introduced into Libya by southward and northward migrating birds, respectively. Most tick species recorded from Libya are of obvious economic importance, yet little is known about their biology, range, incidence, or relation to disease in this area.

This report on Ixodoidea of Libya is part of a two-fold, integrated effort to consolidate and bring up to date available knowledge concerning ticks in northern Africa and the Near East. The aims, systems, and methods of the exploratory phase of this program have been presented earlier (Hoogstraal 1956B, 1958B, 1960). A tangential purpose of this undertaking, in collaboration with the Animal Production Branch of the Food and Agriculture Organization of the United Nations (FAO), is an attempt to obtain more information on ticks parasitizing domestic animals in this area. Species of greatest veterinary importance are usually also incriminated in the epidemiology of human diseases.

Ixodoidea of neighboring Tunisia and Egypt have been reviewed by Colas-Belcour and Rageau (1951) and Hoogstraal and Kaiser (1958E), respectively.

Comparatively little information on Libyan ticks has yet been obtained. Data herein indicate the general distribution and host relations of the species that most probably are of the greatest medical and veterinary importance in these approximately 680,000 square miles between 9° and 25° East longitude and the Mediterrenean and approximately 19° North latitude (figure 1).

Previous literature on the Libyan tick fauna consists of a number of short reports on small collections or single species. Much of it was highly controversial among workers of the period in which it was written and most of the nomenclature is outmoded. Only Libyan data are recorded herein for species otherwise comprehensively treated in Hoogstraal (1956A) or in other very recent papers, to which the reader is referred for further details concerning these forms.

It is hoped that this compilation of information available for Libyan ticks will stimulate interest to learn more about species, distribution, host-predilection, biology, and disease-relationships of ticks in this area.

The following sources of Libyan material were used for this study:

NAMRU-3 COLLECTIONS: Collections made by Mr. Abdel Aziz Salah, NAMRU-3, (Cyrenaica, 27 April to 2 May 1952, during a W.H.O. typhus ourtbreak investigation) and by Mr. Sayed Metwally, NAMRU-3, [Cyrenaica, Fezzan, and Tripolitania, 20 October to 16 December 1955, during study of mammals, supported by Office of Naval Research, United States National Museum, and NAMRU-3 (see Setzer 1957)]. Collections received from Dr. William J. Goodwin (Libyan-American Joint Public Health Service) and Dr. F. Razza (Director of Veterinary Services). Material collected by the writers from Libyan sheep, cattle, and camels being brought into the Western Desert of Egypt.

BRITISH MUSEUM (NATURAL HISTORY): Two specimens of *Ornithodoros foleyi* (=*O. franchinii*) from Libya were studied through the kindness of Mr. E. Browning and Dr. G. Owen-Evans.

[1]From Research Project NM 52 08.03.3. Bureau of Medicine and Surgery, Washington 25, D.C. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Accepted for publication September 11, 1959.

ARGASIDAE

Argas (Argas) persicus (Oken 1818)

THE POULTRY ARGAS OR FOWL TICK

This cosmopolitan parasite was reported from Libya by Zanon (1923), Franchini (1926A, 1927A, 1929A, B), Rondelli (1926), Tonelli-Rondelli (1932A, D), Gaspare (1933), Grimaldi (1934), Zavattari (1934), and Stella (1938). Many records are repetitions of those in earlier papers. Localities from which *A. persicus* has been taken in Libya are: (*Tripolitania*): Misurata, Homs, Sorman, Tagiura, and Tripoli. (*Fezzan*): Gadames and Edri. (*Cyrenaica*): Bengasi, Cufra Oasis, Marada, Tobruch, and Tocra.

In comparison with Libyan records of other tick parasites of domestic animals, the several inland and oasis localities where *A. persicus* has been reported are noteworthy. Dense populations of this argasid also occur in oases and rainless areas of Egypt and Jordan. *A. persicus* appears to be possibly more erratically distributed and less numerous in coastal areas than elsewhere. Indeed, it has been stated that *A. persicus* is absent from coasts in certain parts of the world. Circumstantial details indicate the need for investigation of ecological factors in relation to rate and incidence of *A. persicus* infestation. Libya, with its extensive coastline, gradually more arid inland desert, and isolated oases, would appear to be an ideal region for this study.

For further details concerning *A. persicus*, see Hoogstraal (1956A, pp. 58–74, 862–3).

OTHER *Argas* SPECIES

Because of their rather wide distribution elsewhere in northern Africa, the following *Argas* species probably also occur in Libya: *A.* (*Argas*) *reflexus hermanni* Audouin 1827 (= *A. reflexus* of authors in Africa; see Hoogstraal and Kohls 1960B), *A.* (*Carios*) *vespertilionis* (Latreille 1802) (see Hoogstraal 1958A), *A.* (*Chiropterargas*) *boueti* Roubaud and Colas-Belcour 1933 (see Hoogstraal 1955A), *A.* (*C.*) *confusus* Hoogstraal 1955A, and *A.* (*Secretargas*) *transgariepinus* (White 1846) (see Hoogstraal 1957). An unidentified species of argasid larva was reported from a "turtle dove" at Cufra Oasis by Tonelli-Rondelli (1932D).

Ornithodoros foleyi Parrot 1928

FOLEY'S NORTH AFRICAN TAMPAN

Ornithodoros franchinii sp. nov.; Tonelli-Rondelli 1930B, pp. 113–15, description and illustrations of "adult" from Gadames, Fezzan.

LIBYAN MATERIAL EXAMINED. (*Cyrenaica*): 2♀♀, Bengasi, 11 September 1930, G. Franchini (det. as *O. franchinii* Tonelli-Rondelli, 1930), Nuttall lot 3890 in British Museum (Natural History); one now in Hoogstraal collection, by exchange.

OTHER MATERIAL EXAMINED. (*Egypt*): 2♂♂, 4♀♀, 4NN, numerous reared larvae and nymphs, from Southeastern Desert (see Hoogstraal and Kaiser 1957).

DISTRIBUTION: *O. foleyi* is a North African tick erratically distributed between eastern Algeria and southeastern Egypt. *Algeria:* Ahaggar Mountains (Parrot 1928; Foley 1929). *Libya* (*Fezzan*): Gadames (Franchini 1927A,B, 1929B,C, 1932A,B, 1933A; Tonelli-Rondelli 1930B), Tgutta (Franchini 1933A,D). (*Cyrenaica*): Marsa Lucch and Bardia (Franchini 1932A,B, 1933A,D, 1937; Franchini and Taddia 1930). Cufra Oasis (Tonelli-Rondelli 1932D; Franchini 1932A). *Egypt:* Bir Abraq area of Southeastern Desert (Hoogstraal and Kaiser 1957).

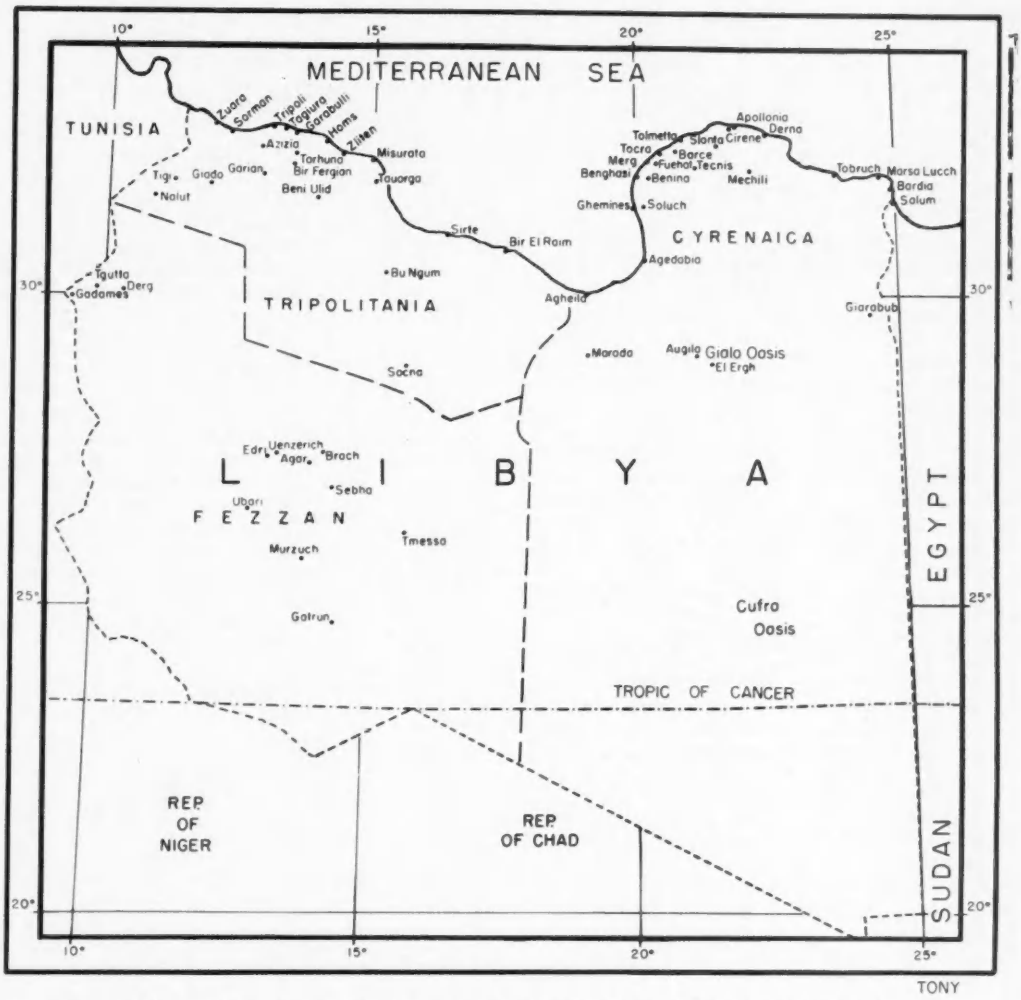
HOSTS: Information concerning hosts of *O. foleyi* is rather confused. Reports from Algeria and Libya are based entirely on remarks by tribesmen living in areas where this tick occurs. Algerian hosts are claimed to be camels and human beings. Libyan hosts are said to be gazelles, human beings, sheep and other domestic animals, and possibly rodents in caves. In Egypt, the only evidence of vertebrate hosts inhabiting the small caves in which these ticks were found indicated the infrequent presence of hedgehogs, geckos, and agamid lizards.

ECOLOGY: *O. foleyi* occurs in coastal semideserts, inland desert wadis, oases, and arid mountains. It appears to be highly adapted to extreme desert conditions and to survival for long periods between blood meals. The Egyptian specimens were buried, together with *Argas brumpti* Neumann 1907, just beneath the surface of sand in very small hillside caves. Algerian and Libyan specimens were reported "from sand" and in sand floors of rocky caves where shepherds find shelter.

LIFE CYCLE: Observations on Egyptian specimens (Davis and Mavros 1956) show that the sex ratio is approximately equal, two to five nymphal instars are required to reach adulthood, egg batches number from 82 to 305 eggs, egg viability is unusually high, larvae require from 5 to 17 days for engorging, and two or more feedings in each stage is the rule rather than the exception. Coxal fluid is seldom passed on the host but flows freely after feeding.

DISEASE RELATIONS: A local lesion similar to a syphilitic chancre was attributed to the bite of *O. foleyi* in Algeria. Franchini (1929B, 1932A,B; 1933D) and Franchini and Taddia (1930) claimed similar sequelae in Libya, as well as "tick-bite fever" as a result of *foleyi* bites. The actual role of this tick in relation to human injury and disease should be exceedingly interesting to investigate more thoroughly. Spirochetes were not recovered from Egyptian specimens.

REMARKS: A new species very closely related to *O. foleyi* has recently been found in a porcupine



TONY

FIG. 1.—Map of Libya, showing Provinces and most of collecting localities mentioned in text.

burrow in Pretoria District, Union of South Africa (Theiler, in press).

Warburton (1933) compared this species with *Argas brumpti* and discussed certain implications of its slightly modified lateral integument from the taxonomic viewpoint.

According to Franchini and Tonelli-Rondelli (loc. cit.) the local name of this tick is *tddia* (*teddia*) or *tbbia* (*tebbia*) in Tripolitania and *a rambda* in Cyrenaica.

Ornithodoros savignyi (Audouin 1827)

THE EYED TAMPAN

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 3♂♂, 2♀♀, 7NN, cattle, Dal Mercata, Azizia district, Veterinary Department. 1♂, ground at water hole, Tigi, Dr. W. J. Goodwin.

OTHER LIBYAN RECORDS: The following collecting localities were reported by Tonelli-Rondelli. (*Fezzan*): Edri and Gadames (1932B) and Tmessa, Sciaua, Borg, Mezzem, and Uenzerich (1935). (*Cyrenaica*): Marada and Augila (1932A,D).

REMARKS: As noted below under *O. moubata*, in the section on other *Ornithodoros* species, a bitter controversy raged for some years between Franchini, Gaspare, Garibaldi, and Zavattari over the question of whether *O. savignyi* or *O. moubata* is present in Libya. Franchini in numerous publications claimed to have recovered pathogenic spirochetes from *O. moubata* from

Libya. Little credence has generally been accorded these reports, which may actually refer to *O. foleyi* or *O. tholozani*. *O. savignyi* is probably widely distributed in Libyan oases and deserts, but exact details are meagre. For further information concerning *O. savignyi*, see Hoogstraal (1956A, pp. 191–9, 867).

Ornithodoros tholozani Laboulbène and Megnin 1882

THE PERSIAN RELAPSING FEVER TAMPAN

Coghill et al. (1947) described relapsing fever in British troops in the area between Bengasi, Apollonia, and Tobruch, Cyrenaica, during World War II. They also reviewed earlier literature on this disease in Libya, much of it of uncertain accuracy. Ticks from Tobruch were definitely identified as *O. tholozani* by Professors S. Adler and O. Theodor, and were shown to be spirochete-infected. It appears probable that *O. tholozani* may actually have been the species involved in earlier reports of various species of *Ornithodoros* in relation to relapsing fever in Libya. Confirmation of the fact that this Middle Eastern argasid species ranges into northern Africa was received when spirochete-infected populations of *O. tholozani* were more recently discovered in the Western Desert of Egypt close to the Libyan border (Davis and Hoogstraal 1956). Literature on this very important species has been reviewed by Anastos (1957). Thorough investigation of this species in relation to human and animal diseases in Libya is indicated.

OTHER *Ornithodoros* SPECIES AND NAMES

"*Ornithodoros annulatus*" Grimaldi 1934, a *nomen nudum*, is probably a misprint for *Boophilus annulatus*.

Ornithodoros arenicolous Hoogstraal 1953, fairly common in Egyptian coastal deserts as far as the Libyan border, has recently been determined (Colas-Belcour and Morel 1958) also to occur in Tunisia. The range of this species, therefore, probably includes coastal and possibly other areas of the Libyan Kingdom.

Ornithodoros d. delanoëi Roubaud and Colas-Belcour 1931, was originally described from Morocco. In Egypt, it is sparsely but widely distributed in desert caves and dry burrows in cultivated areas near the desert edge (Hoogstraal 1955C). Its presence in Libya is to be expected.

Ornithodoros erraticus Lucas 1849, has not been reported from Libya. This tick is so common throughout northern Africa that absence of Libyan records obviously reflects lack of search in mammal burrows rather than discontinuous distribution. It is a characteristic parasite of many wild animals in the Egyptian littoral desert as far west as the Libyan frontier and is commonly infected with the spirochete *Borrelia crocidurae* (Hoogstraal et al. 1954; Davis and Hoogstraal 1954).

Ornithodoros lahorensis Neumann 1908, mentioned by Franchini in practically all of his papers on Libya, as well as by Grimaldi (1934) and Garibaldi (1935), appears most probably to refer entirely or in part to *O. foleyi*.

Ornithodoros moubata (Murray 1877) was recorded by Franchini (1932A,B, 1933A,B,C, 1934B, 1935A) from Tripolitania. Gaspare (1933, 1934) and Garibaldi (1935) accepted these reports but Zavattari, who first reported *O. savignyi* from Libya (1930), asserted (1932, 1933, 1934) that *O. moubata* is absent there—an opinion with which we are certainly inclined to agree.

IXODIDAE

Boophilus annulatus (Say 1821)

THE TEXAS FEVER TICK

B. annulatus appears to be probably quite rare and localized in Libya. Tonnelli-Rondelli did not mention it and we have seen no Libyan specimens. Franchini reported "*Rhipicephalus annulatus*" from Gariaun, Tripolitania (1929B), and in Cyrenaica from Bengasi and Gialo (1929A) and Giarabub (1927A, 1929C). These records were reiterated by Grimaldi (1934) and Garibaldi (1935); the latter added Soluch, Cyrenaica, to the list. For further details on this species see Hoostraal (1956A, pp. 292–304).

Haemaphysalis otophila Schulze 1918

THE NEAR-EASTERN SHEEP AND GOAT HAEMAPHYSALID

Haemaphysalis otophila schulzei subsp. nov.; Rondelli 1926, pp. 51–54, figs. 2 and 3; description and illustration of ♂♂ and ♀♀ from Merg and Tolmetta (Cyrenaica). We are unable to differentiate samples from Libyan populations from European and Asiatic material.

Haemaphysalis sulcata otophila Schulze 1918; Tonelli-Rondelli 1932E; 1♂, 6♀♀, from goat, Agedabia (Cyrenaica).

LIBYAN MATERIAL EXAMINED. (*Cyrenaica*): 2♂♂, donkey, Bengasi, April 1952, A. A. Salah. 95♂♂, 100♀♀, goats, Slonta; 32♂♂, 20♀♀, sheep, Benina; November, 1955, S. Metwally.

OTHER MATERIAL EXAMINED. *Egypt:* 16♂♂, 350♀♀ from approximately 100 Libyan sheep at Salum frontier station, en route to El Hammam market, 23 October 1953, H. Hoogstraal and M. N. Kaiser. In addition, our collections contain approximately 400 specimens from Italy, Turkey, Jordan, Iraq, and U.S.S.R. We have also seen material from Bulgaria and Palestine, in the Rocky Mountain Laboratory collection.

DISTRIBUTION: The center of distribution of *H. otophila* appears to be in the Black Sea area. On the African continent it is known only from Cyrenaica. This species may have once been established in northern Egypt but it is now not present here. Libyan populations of *H. otophila* possibly result from adventitious introductions from nearby Europe, where it occurs in Italy, Greece, Yugoslavia, Hungary, Czechoslovakia,

Rumania, and Bulgaria. The Asiatic range of *H. otophila* includes Palestine, Turkey, northern Iraq, southern Ukrainia, Crimea, eastern Transcaucasia, the Black Sea littoral of western Transcaucasia, and western Turkmenia; it is especially numerous in Ciscaucasia and Daghestan.

HOSTS: In the U.S.S.R., where *H. otophila* has been much studied, Pomerantzev (1950) lists sheep and goats as the most common hosts of adults, and any other domestic animals, cattle, camels, horses, donkeys, buffalos, and dogs as secondary hosts. Human beings may also be attacked. Immature stages feed on hedgehogs, rodents, hares, birds, lizards, and human beings. In Turkey, some hosts of immature stages were found to be hedgehogs, jerboas, jirds, mole rats, hamsters, and various genera of field mice (Hoogstraal 1959). Sheep and goats appear to be especially heavily parasitized in Cyrenaica; hosts of immature stages are unknown here but probably include hedgehogs and rodents and to a lesser degree lizards and birds.

ECOLOGY: *H. otophila* is obviously well adapted to ecological conditions in Cyrenaican coastal deserts. It is impossible to evaluate reasons for lack of Tripolitanian records. Fertilized, greatly engorged females of this species are commonly introduced into the Western Desert of Egypt, as far east as Alexandria, on sheep imported from Libya. However, they do not survive in Egyptian deserts, which are more arid than those of coastal Cyrenaica.

As summarized by Pomerantzev (1950), Russian populations of *H. otophila* inhabit lowland and elevated steppe zones and range into lower mountain forest zones up to 1250 meters altitude. Possibly owing to sensitivity of larvae and nymphs to low relative humidity, this species is uncommon in semidesert regions. Large numbers may occur on a single animal even though the species may otherwise not be common in the area.

LIFE CYCLE: *H. otophila* is a three-host tick whose adults feed chiefly from late summer to spring and are most numerous in autumn. Published records and our experience outside of the U.S.S.R. confirm reports of Russian workers in this respect. The large number of Libyan females taken in October and November indicate that fall is an active breeding period in Cyrenaica. Immature stages are chiefly summer parasites.

The female commences oviposition from 3 to 49 days (average 8 days) after completing feeding, oviposits for 12 to 20 days (average 18 days), and dies from 5 to 30 days afterwards. Larvae hatch after 25 to 36 days, may survive unfed for 3 to 8 months, and engorge for 2 to 7 days (average 4 days). From 15 to 28 days afterwards they molt to nymphs, which can survive unfed for 2½ months. Nymphs feed for 2 to 8 days (average 3 days), and molt to adults in 17 to 26 days (average 18 days). Unfed adults may live for over 6 months. The female feeds from 3 to 14 days and may deposit up to 3,000 eggs (Pomerantzev 1950).

DISEASE RELATIONS: This species is said to be a vector of *Babesia ovis* of sheep.

REMARKS: Following Tonelli-Rondelli's (1932E) report of both "*H. sulcata otophila*" and "*H. otophila schulzei*" in Libya, reviewers of this fauna used these specific and subspecific designations in a number of combinations, often as quadrinomials.

OTHER *Haemaphysalis* SPECIES

Haemaphysalis e. erinacei Pavesi 1884. Although this parasite of hedgehogs has not been recorded from Libya, it is known from northwestern Africa and is quite common in the Mediterranean littoral semidesert of western Egypt (Hoogstraal 1955B). We have little hesitation in assuming that it will be found to occur in numerous localities of northern Libya.

Haemaphysalis l. leachii Audouin 1827. A single male of the Yellow Dog-Haemaphysalid was reported, from an unknown host, at Merg (Cyrenaica) by Rondelli (1926), who suggested that it may have been imported from the south or east. Garibaldi (1935) listed Tecnis, Cyrenaica, as the only locality for *leachii* in Libya. We are not aware of substantiated reports of this species from northern Africa west of the Nile Delta (see Hoogstraal 1958C). While it is possible that isolated populations exist in Cyrenaica, incorrect identification of the single available specimen (possibly actually *H. e. erinacei* Pavesi 1884) needs to be considered. This record was repeated by Franchini and others in a number of papers.

Haemaphysalis punctata Canestrini and Fanzago 1887. According to Rondelli (1926), a female of this species was collected in Wadi Derna (Cyrenaica) between December and March. Garibaldi (1935) listed *H. punctata* from Beni Ulid and Zuara in Tripolitania and from Apollonia, Cirene, Derna, and Marada in Cyrenaica. Assuming these identifications to be correct, they would indicate isolated populations of *H. punctata*, which ranges widely through Europe, the Near East, and the U.S.S.R. We have not found *H. punctata* in Egypt outside of Sinai. It is not known from Tunisia or Morocco but small populations exist in Algeria. The actual status of this species in Libya is uncertain.

Hyalomma (Hyalomma) anatolicum anatolicum Koch 1844

THE SMALL ANATOLIAN HYALOMMA

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 1 ♀, camel, Giado. 3 ♂♂, 3 ♀ ♀, sheep; 2 ♂♂, camel; Sirte. 3 ♂♂, 4 ♀ ♀, sheep, Tarhuna. 4 ♂♂, 4 ♀ ♀, horse, Azizia. 2 ♂♂, sheep, Misurata. 2 ♀ ♀, on ground, resting place of sheep, Wadi Caam. 1 ♂, ground at water hole, Tigi. Collected by Veterinary Department and Dr. W. J. Goodwin.

(*Cyrenaica*): 3♂♂, 1♀, sheep, Gardinia, 29 April, 1952, A. A. Salah. 3♂♂, 3♀♀, goats; 1♀, sheep; 2♀♀, camels; 3 mi. w. of Agheila; 27 October 1955; S. Metwally. 1♂, 3♀♀, sheep; 5♂♂, 1♀, cattle; Benina, 5 November 1955, S. Metwally. 37♂♂, 31♀♀, camels, Ghemines, 29 October 1955, S. Metwally.

REMARKS: See Remarks under *H. anatolicum* subspp. (below).

Hyalomma (Hyalomma) anatolicum excavatum Koch 1844

THE LARGE ANATOLIAN HYALOMMA

H. anatolicum zavattarii n. subsp; Tonelli-Rondelli 1935, pp. 240–2, fig. 1, description and illustration of ♂ from Murzuch, Fezzan.

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 1♀, sheep; 3♀♀, camel, Zuara. 2♂♂, 11♀♀, sheep; 8♂♂, 7♀♀, camels; Sirte. 5♂♂, 8♀♀, camel, Beni Ulid. 2♂♂, 1♀, camel, Azizia. 3♂♂, 3♀♀, camel; 10♂♂, 12♀♀, sheep; 3♀♀, goats; Misurata. 2♂♂, 7♀♀, camel, Giado. 5♀♀, camel, Garian. 1♂, camel; 1♀, 1 inch below surface of soil around well where animals drink; Bir El Raim, 2 July 1958. Collected by Veterinary Department and Dr. W. J. Goodwin.

(*Cyrenaica*): 1♂, 4♀♀, sheep; 4♂♂, 4♀♀, cattle; Benina, 5 November 1955, S. Metwally. 19♂♂, 4♀♀, camels Ghemines, 29 October 1955, S. Metwally. 6♂♂, 8♀♀, camels; 2♂♂, on ground; 4♂♂, 2♀♀, goats; 3 to 5 mi. w. of Agheila; 27 October 1955, S. Metwally. 1♂, 3♀♀, goats, Gardinia, April, 1952, A. A. Salah.

REMARKS: See Remarks under *H. anatolicum* subspp. (below).

Hyalomma (Hyalomma) anatolicum subspp. intermediate

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 3♂♂, 5♀♀, horse, Sirte. 3♂♂, 4♀♀, sheep, Tarhuna. 2♂♂, 2♀♀, cattle; 2♂♂, 1♀, horse; 5♂♂, 6♀♀, camel; Azizia district. 3♂♂, 1♀, camel, Garabulli. Collected by Veterinary Department.

REMARKS: The *H. anatolicum* complex has recently been extensively reviewed in this journal (Hoogstraal and Kaiser 1959). Further remarks are unnecessary here except to call attention to the fact that of the 277 Libyan specimens examined, 37, or approximately 1.3%, were intermediate between the two subspecies and all of these were from Tripolitania.

H. depressum Schulze 1918, reported from Libya by Rondelli (1926) and others, probably refers to one of the subspecies of *H. anatolicum*.

Hyalomma (Hyalomma) dromedarii Koch 1844

THE CAMEL HYALOMMA

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 3♂♂, 2♀♀, camels, Derg. 3♂♂, 3♀♀, under tree, 23 mi. w. of Bu Ngem. 64♂♂, 22♀♀, camels, 45 mi. s. of Socna. 14♂♂, 17♀♀, camels, Misurata. 8♂♂, 3♀♀, camels, 1♀, horse, Sirte. 1♂, 1♀, camel, 1♀, horse, Azizia. 2♂♂, 3♀♀, camel, Giado. 2♀♀, Zuara. 5♂♂, camel, Beni Ulid. 2♀♀, camel, Zuara. 1♀, horse, Ben Ghescir. 1♂, Bir Fergian. Collected by Veterinary Department and Dr. W. J. Goodwin.

(*Cyrenaica*): 16♂♂, 7♀♀, camels, Ghemines; 3♀♀, camel, Agheila; October, 1955, S. Metwally.

OTHER LIBYAN RECORDS. Franchini and Cadeddu (1927) reported *H. dromedarii* from Giarabub, Cyrenaica, in relation with a trypanosome infection of camels. This tick has been listed from numerous localities in all previous lists of Libyan ticks. While it is undoubtedly widely spread in this area, frequent misidentification of *Hyalomma* species in the period before World War II casts doubt on the accuracy of previous data. For further information concerning this species, see Hoogstraal (1956A, pp. 420–34, 876–8).

Hyalomma (Hyalomma) franchinii Tonelli-Rondelli 1932

THE NORTH AFRICAN LIZARD HYALOMMA

Hyalomma tunesiacum franchinii n. subspp.; Tonelli-Rondelli 1932C, pp. 121–2; description and illustration of ♂ from Tauroga Oasis, Tripolitania. For further taxonomic details, see Hoogstraal (1956A, pp. 880–3) and Hoogstraal and Kaiser (1958A, D).

LIBYAN MATERIAL EXAMINED. (*Tripolitania*), 2♂♂, camel and sheep, Tauroga Oasis, Spring, 1931, *Cotypes* (see Hoogstraal and Kaiser, 1958B). 2♂♂, 1♀, camel, Sirte, 6 January 1958, Dr. W. J. Goodwin. 4♀♀, camel, Buagiah, Garabulli, Veterinary Department. (*Cyrenaica*): 1♂, tortoise; 1♀, camel; Ghemines, 29–30 October 1955, S. Metwally.

OTHER MATERIAL EXAMINED. *Tunisia:* 2♀♀, camels, Khereldine, August, 1958, Dr. M. L. Tarizzo. *Egypt:* Approximately 2,000 specimens collected in nature and laboratory reared.

REMARKS: *H. franchinii* is a North African tick ranging from Tunisia into Sinai. It has not previously been recorded from Tunisia and we are grateful to Dr. M. L. Tarizzo of the World Health Organization for sending material from there. In spite of the very few available records from Libya, we suspect that *H. franchinii* might be quite numerous in certain areas of the Kingdom. Libyan camels arriving at the El Hammam market in the Western Desert of Egypt are heavily infested, especially during winter and spring when engorged females are numerous on these animals. *H. franchinii* is fairly common in the western littoral desert of Egypt and along the margin of the Nile Valley and Delta of Lower Egypt but less so in the Eastern Desert and Sinai.

Northcentral and northeast African desert margins, littoral deserts, and lowland and mountain

semideserts, inhabited by lizards and domestic animals, are ecological areas in which *H. franchinii* occurs.

Immature stages parasitize lizards and, less commonly, rodents. Adult hosts are camels, cattle, sheep, and donkeys. A single specimen, taken on a tortoise, may indicate primitive host predilection of adults of this species, a question worthy of investigation. Adults feed on man under experimental conditions. *H. franchinii* is a three-host tick. Immature stages are found on lizards throughout the year. Adults appear to be most common and to breed most actively during the cooler months of the year.

This species was redescribed and reillustrated by Hoogstraal and Kaiser (1958A,D), who also included additional data on the life-cycle and hosts.

Hyalomma (Hyalomma) impeltatum Schulze and Schlottke 1930

KRATZ'S HYALOMMA

Hyalomma fezzanensis n. sp.; Tonelli-Rondelli 1935, pp. 242–4, description and illustrations of 3♂♂ and 5♀♀ from Ubari, Fezzen.

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 1♂, sheep, Tarhuna. 2♀♀, cattle; 2♂♂, camel; 1♂, horse; Azizia district. 1♂, horse, Cabila Amain Derg, Ben Ghescir. 15♂♂, 4♀♀, camels, Buagiah, Garabulli. 14♂♂, 25♀♀, sheep; 11♂♂, 21♀♀, camels; 2♂♂, 2♀♀, horse; Sirte district. 1♂, camel, Beni Ulid. 6♂♂, 8♀♀, sheep, Zuara. 1♀, camel, Giado. 1♂, 1♀, camel, Bir Fergian. 4♂♂, 3♀♀, camel, Misurata. 1♂, 3♀♀, camel, Garian. Most of the above specimens were sent by Dr. W. J. Goodwin and the Veterinary Department. 1♂, camel, 45 mi. s. of Socna; 1♂, camel, Misurata; 1♂, under a tree, 23 mi. w. of Bu Ngem; October-November, 1955, S. Metwally.

(*Fezzan*): 7♂♂, 9♀♀, camels, Derg; December, 1955, S. Metwally.

(*Cyrenaica*): 1♂, 3♀♀, camels; 1♂, 1♀, sheep; 1♂, goat; 3 mi. w. of Agheila; October, 1955. S. Metwally.

REMARKS: *H. impeltatum* appears to be quite common and widely distributed in Libya. Closer examination of sheep and goats will probably reveal an even greater incidence than indicated by present data, which are chiefly from camels. For further details, see Hoogstraal (1956A, pp. 452–60), and Hoogstraal and Kaiser (1958C).

Hyalomma (Hyalomma) marginatum rufipes Koch 1844

THE HAIRY HYALOMMA

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 1♀, camel, Buagiah, Garabulli district; 1♂, Cabila Miamin, Azizia district; Veterinary Department. (*Cyrenaica*): 1♂, camel, Ghemines, 29 October 1955, S. Metwally.

REMARKS: See Remarks under *H. marginatum turanicum* (below).

Hyalomma (Hyalomma) marginatum turanicum Pomerantzev 1946

THE TURANIAN HYALOMMA

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 1♀, camel, Garian, 15 June 1957, Veterinary Department.

REMARKS: After exhaustive study of large numbers of *H. marginatum, rufipes*, and *turanicum* from numerous areas in Europe, Africa, and Asia, it has become apparent that each of these forms is a geographical but closely related subspecies. Characteristic hosts of immature stages are birds, and the distributional pattern of each is complicated by more or less permanent establishement of populations outside the normal range of the subspecies through the agency of migrating birds. Introduction on domestic animals imported for slaughter or breeding appears to be another source of exceptional foci, and hybridization between local and "foreign" populations undoubtedly occurs in some areas. The study of this subject is nearing completion and will be presented shortly. Hoogstraal and Kaiser (1958B) have demonstrated the frequent passage of *rufipes*-infested migrant birds over Cairo, Egypt, as well as a single incidence of introduction of *marginatum* into Egypt on a bird arriving from Europe during the autumn migration. In the past 2 years a considerable volume of additional data concerning tick-infested migrating birds have been obtained. For further details concerning the forms *marginatum, rufipes*, and *turanicum*, see Hoogstraal (1956A, pp. 465–90, 527–32, 887–90) and Pomerantzev (1950, pp. 214–19).

It is probable that specimens identified by Tonelli-Rondelli (1930A), and repeated by other reviewers, as *H. marginatum balcanicum* Schulze and Schlottke 1930, were either *rufipes* or *turanicum*. We consider the name *balcanicum* to be a *nomen nudum*. Tonelli-Rondelli's specimens were from Tripoli and Bengasi. The typical subspecies, *marginatum*, is not known from Libya and appears to be only adventitious in Egypt. To the west, in Algeria and Morocco, scattered populations of *marginatum* occur.

Hyalomma spp. (immature stages)

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 1L, sheep, Sirte. 9LL, 3NN, *Gerbillus pyramidum aureus* Setzer, 1956, 12 km. w. of Zliten, 21–22 October. 1N, *Gerbillus pyramidum favillus* Setzer, 1956, 2 km. e. of Sirte, 24 October. 28LL, 7NN, *Gerbillus g. gerbillus* (Olivier, 1801), 5 to 10 km. s. of Socna, 28–29 November. 1L, *Meriones libycus confalonierii* de Beaux, 1931, 12 km. w. of Zliten, 22 October. 8LL, 1N, *Meriones libycus caudatus* Thomas, 1919, Bir Fergian, 10 km. s. of Socna, 29 November. 1N,

Table 1.—Immature stages of ticks recovered from small wild mammals (except bats) in Libya

Number mammals examined	*Hyalomma* spp.		*Rhipicephalus s. sanguineus*		Mammal form
	Larvae	Nymphs	Larvae	Nymphs	
1	0	0	0	1	*Erinaceus (A.) a. algirus* (Hedgehog)
1	0	1	0	0	*Hemiechinus auritus libycus* (Hedgehog)
1	110	16	0	0	*Lepus barcaeus* (Hare)
1	0	0	0	0	*Microtus mustersi* (Vole)
10	4	1	26	0	*Gerbillus eatoni* (Lesser gerbil)
20	37	10	0	0	*Gerbillus g. gerbillus* (Lesser gerbil)
14	9	3	24	6	*Gerbillus pyramidum aureus* (Greater gerbil)
20	0	1	0	0	*Gerbillus pyramidum favillus* (Greater gerbil)
48	0	0	0	2	*Gerbillus pyramidum tarabuli* (Greater gerbil)
35	0	0	2	0	*Gerbillus (D.) campestris dodsoni* (Dipodil gerbil)
9	1	0	0	0	*Gerbillus (D.) dasyurus vivax* (Dipodil gerbil)
1	0	0	0	0	*Gerbillus (D.) h. henleyi* (Dipodil gerbil)
1	6	1	0	0	*Pachyuromys duprasi natronensis* (Fat-tail sand rat)
7	0	1	0	0	*Meriones crassus tripolius* (Jird)
1	8	1	0	0	*Meriones libycus caudatus* (Jird)
8	16	18	0	1	*Meriones libycus confalonierii* (Jird)
2	1	1	0	0	*Meriones shawi azizi* (Jird)
11	35	49	43	11	*Meriones shawi shawi* (Jird)
2	2	6	1	0	*Psammomys obesus tripolitanus* (Fat sand-rat)
1	0	0	0	0	*Psammomys v. vexillaris* (Fat sand-rat)
17	0	0	0	0	*Mus musculus* subsp. (Mouse)
2	0	0	0	0	*Acomys cahirinus viator* (Spiny mouse)
1	0	0	0	0	*Eliomys quercinus cyrenaicus* (Dormouse)
18	6	4	6	2	*Jaculus o. orientalis* (Greater jerboa)
3	4	0	1	1	*Allactaga tetradactyla* (Four-toed jerboa)
1	0	0	0	0	*Ctenodactylus gundi vali* (Gundi)
1	0	0	0	0	*Canis aureus algirensis* (Jackal)
8	0	0	0	0	*Gazella d. dorcas* (Gazelle)
Total 245	219	113	103	24	No. mammal forms examined: 28

Meriones crassus tripolius Thomas, 1919, 5 km. s. of Socna, 28 November.

(*Cyrenaica*): 1N, shrike (bird), 30 km. s. of Bengasi. 1N, *Gerbillus eatoni* Thomas, 1902, 5 km. w. of Agheila, 28 October. 4LL, *G. eatoni*, 4 km. w. of Ghemines, 30 October. 8LL, 3NN, *G. g. gerbillus*, 5 km. w. of Agheila, 27 October. 1L, *G. g. gerbillus*, 65 km. wnw. of Agheila, 26 October. 1L, *Gerbillus* (*Dipodillus*) *dasyurus vivax* (Thomas, 1902), 5 km. w. of Agheila, 28 October. 2LL, 6NN, *Psammomys obesus tripolitanus* Thomas, 1902, Ghemines, 31 October. 1N, burrow of *P. o. tripolitanus*, 5 km. w. of Agheila, 27 October. 1L, 1N, *Meriones shawi azizi* Setzer, 1956, 5 km. se. of Derna, 9 November. 35LL, 49NN, *Meriones s. shawi* (Duvernoy, 1842), Ghemines, 29–31 October. 15LL, 18NN, *Meriones libycus confalonierii*, 5 km. w. of Agheila, 27–28 October, 6LL, 1N, *Pachyuromys duprasi natronensis* de Winton, 1903, 64 km. wnw. of Agheila, 26 October. 6LL, 4NN, *Jaculus o. orientalis* Erxleben, 1777, Ghemines, 29–31 October. 4LL, *Allactaga tetradactyla* (Lichtenstein, 1823), Ghemines, 31 October. 1N, *Hemiechinus auritus libycus* (Ehrenberg, 1833), Wadi El Ahmar, 40 km. se. of Bengasi, 16 November. 110LL, 16NN, *Lepus barcaeus*, Wadi Ahmar, 40 km. se. of Bengasi, 15 November.

REMARKS: The immature *Hyalomma* ticks listed above were obtained by Sayed Metwally of the NAMRU–3 Medical Zoology staff between 20 October and 16 December 1955. Their hosts have been studied by Setzer (1957). In table 1, the total number of animals examined during this period and the number of immature *Hyalomma* ticks removed from them are summarized. It is especially noteworthy that these collections were made in early winter. Examination of small wild animals at other seasons would probably reveal a higher incidence and rate of infestation. The data for large numbers of these ticks on the single hare examined, the consistent infestation of jirds, and the frequent infestation of some forms of gerbils are similar to those we have obtained in other areas of northern Africa and Arabia. Unfortunately, criteria for differentiating species of immature stages of hyalommas are not yet known.

OTHER *Hyalomma* SPECIES

H. (*Hyalommasta*) *aegyptium* (Linnaeus 1758), as used in all previous reports of Libyan ticks, refers entirely or in large part to other species from domestic animals. No definite data concerning the true *H. aegyptium* of tortoises (*Testudo* sp.) are available from Libya, but this tick is common on tortoises in Tunisia (Colas-Belcour and Rageau 1951) and might be fairly widely distributed in Libya. To the east, in Egypt,

tortoises are now extinct except near the Libyan and Palestinian borders, where they are not infested by *H. aegyptium*. For further details concerning this species see Hoogstraal (1956A, pp. 513–16).

H. (*Hyalomma*) *detritum* Schulze 1919 (=*H. mauritanicum*) was reported from Giarabub, Cyrenaica, by Franchini (1927) and Garibaldi (1935). While it is entirely possible that isolated populations of *H. detritum* occur in Libya, we tentatively consider this to be a questionable record owing to confusion in *Hyalomma* species identification in the period before World War II. For additional information on *H. detritum*, see Hoogstraal (1956A, pp. 403–19, 871–6).

Ixodes festai Rondelli 1926

FESTA'S NORTH AFRICAN RUSSET TICK

DISTRIBUTION: *I. festai*, of the western Mediterranean region, is known to inhabit the following localities in southwestern Europe and northwestern Africa. *Libya* (*Cyrenaica*): Derna (Rondelli 1926). *Tunisia:* Zriba, Enfidaville (Colas-Belcour and Rageau 1951). *Morocco:* Nefifik Forest near Casablanca (Arthur 1957, 1958. Blanc and Bruneau 1956, 1958). *France:* Bouilly (Loiret), Bois de Boulogne (Seine), Fontainebleau (S. et M.), Orsay (S. et O.), Ille de Loch (Glenans, Fin.), Cadarache (B. du Rh.), Aubenas (Ardeche), and Maureillas (Pyr. Or.) (Morel, in press). *Spain:* Barcelona (Gil Collado 1936, 1948, as *Ixodes ventalloi*).

HOSTS: European rabbits are possibly favorite hosts of all stages of *I. festai*. Females are also known from ground-feeding birds and hedgehogs. Lizards and a variety of rodents are common hosts of immature stages but seldom if ever harbor females of this species.

Rabbits, *Oryctolagus cuniculus* subspp., are common hosts of larvae, nymphs, and females in Morocco and France. Females have been taken from Barbary partridges, *Alectoris b. barbata* (Reichenow 1896) in Libya and Tunisia, and from the little owl, *Aethene noctua* subsp., in Spain. In France, the European fence lizard, *Lacerta a. agilis* Linnaeus 1758, is a host of nymphs. In Morocco, the following rodents and carnivores have been recorded as hosts of immature stages: Northwest African garden dormouse, *Eliomys quercinus munbyanus* (Pomel 1856); Barbary striped mouse, *Lemniscomys barbarus* subsp.; house mouse, *Mus musculus spretus* Lataste 1883; domestic rat, *Rattus rattus* subsp.; dipodil gerbil, *Gerbillus* (*Dipodillus*) *campestris* subsp.; common field mouse, *Apodemus sylvaticus hayi* (Waterhouse 1837); fox, *Vulpes vulpes barbara* (Shaw 1800) (=*V. v. acaab* Cabrera 1916); and weasel, *Mustela nivalis numidica* (Pucheran 1885). The following lizards are also infested in Morocco: eyed skink, *Chalcides ocellatus* (Forskal 1775); Bibron's agama, *Agama bibroni* A. Dumeril 1851; Algerian skink, *Eumeces a. algeriensis* (Peters 1864); and plated lacertid, *Psammodromus a. algirus* (Linnaeus 1758).

ECOLOGY: Immature stages of *I. festai*, which are possibly more susceptible to environmental factors than adults, appear to be adapted to animal burrows in open, dry forests and in certain semidesert areas, as well as to rocky niches, in the comparatively arid conditions of the western Mediterranean area.

Adults have been found on hosts together with *Rhipicephalus s. sanguineus* (Latreille 1806) and *Hyalomma lustanicum* Koch 1844 (for details of the latter species, see Hoogstraal and Kaiser 1959).

LIFE CYCLE: Details of the life cycle were reported by Blanc and Bruneau (1958) and by Arthur (1958) from data furnished by Dr. J. Bruneau. Mating occurs on the host, while the female is feeding (Bruneau, personal correspondence). Females and nymphs appear on rabbits during the cold season, beginning in October, and become more numerous from November through January. In February their numbers greatly diminish and few if any are found until October. Larvae feed chiefly in April.

At a temperature of 22° C. and 80% RH or more, engorged females oviposit from 15 to 30 days after finishing feeding and eggs hatch from 45 to 60 days later. Larvae engorge in 3 to 7 days at 22° C., and in 3 or 4 days at 25°–26° C. Molting occurs after 21 to 30 days. Nymphs engorge in 4 to 9 days at 22° C. but at 26° all engorge in 4 days. At 26° C. they molt after 37 or 38 days. Most females on rabbits disengage from the host after 7 days but some remain attached for 11 to 13 days.

Larvae and nymphs attach to the head, lower jaw, and axillae of lizards and under the lower jaw and in the ears of rodents. One to 10 specimens are usually found on rodents, 20 on certain lizards, and 45 to 60 on rabbits. On one *Eliomys*, 117 immature specimens were found.

REMARKS: Arthur (1957) redescribed and reillustrated the male and female and (1958) provided the same data for all stages.

This species resembles the North American leporid parasite *I. dentatus* Marx 1899, *I. spinipalpis* Hadwen and Nuttall 1916, and *I. neotomae* Cooley 1944. Arthur (personal correspondence) believes that this is an instance of parallel evolution rather than of close species relationship.

OTHER *Ixodes* SPECIES

Ixodes ricinus (Linnaeus 1758) was reported from Bengasi, Cyrenaica, by Zanon (1923), Garibaldi (1935), and others. This record is questionable.

Rhipicephalus s. sanguineus (Latreille 1806)

THE KENNEL TICK

LIBYAN MATERIAL EXAMINED. (*Tripolitania*): 2♀♀, camel, Giado. 9♂♂, 21♀♀, sheep;

2♀♀, camel, Sirte. 17♂♂, 23♀♀, camel; 4♂♂, 19♀♀, goats, Misurata. 1♀, ground at water hole, Tigi. Above material from Veterinary Department. 4♂♂, 2♀♀, goat; 2♂♂, 1♀, sheep; Bir El Raim, 2 July 1958, Dr. W. J. Goodwin. 1N, *Meriones libycus confalonierii*; 24LL, 6NN, *Gerbillus pyramidum aureus* (13 hosts); 12 km. w. of Zliten, 22 October 1955, S. Metwally.

(*Fezzan*): 2NN, *Gerbillus pyramidum tarabuli*, 5 km. nw. of Sebha, 3 December 1955, S. Metwally.

(*Cyrenaica*): 3♀♀, sheep; 6♂♂, 4♀♀, goats; Gardinia, 29 April 1952, A. A. Salah. All the following collected by S. Metwally between 20 October and 16 December 1955. 1♂, sheep, Benina. 2♂♂, *Hemiechinus auritus libycus*; 1♂, 1♀, 1N, *Erinaceus* (*A.*) *algirus algirus*; 4♂♂, 1♀, *Lepus barcaeus*; Wadi Ahmar, 40 km. se. of Bengasi. 26LL, *Gerbillus eatoni*, 4 km. w. of Ghemines. 2LL, *Gerbillus* (*Dipodillus*) *campestris dodsoni*, 5 km. se. of Derna. 43LL, 11NN, *Meriones shawi shawi*; 1L, *Psammomys obesus tripolitanus*; 6LL, 22NN, *Jaculus orientalis orientalis*; 1L, 1N, *Allactaga tetradactyla*; Ghemines.

REMARKS: For further details on wild animals examined for ticks in Libya, see section on *Hyalomma* spp. (immatures) above, table 1, and Setzer (1957). Collecting during warmer months of the year will probably yield data indicating a higher rate and incidence of infestation of wild animals by both immature and adult stages of the kennel tick.

Rondelli (1926) reported this species to be widely distributed in Cyrenaica on horses, foxes, hares, and hedgehogs. The NAMRU collection of two nymphs from a gerbil near Sebha provides the only evidence of the occurrence of *R. s. sanguineus* in Fezzan outside of the Gadames area.

All previous reviewers of the Libyan tick fauna list this species. Areas and localities from which it has been reported are: (*Tripolitania*): Beni Ulid, Giado, Misurata, Nalut, Tagiura, Tarhuna, Tauorga, Tripoli, and Zuara. (*Fezzan*): Gadames. (*Cyrenaica*): Agedabia, Apollonia, Barce, Bengasi, Cirene, Cufra Oasis, Derna, Fuehat, Ghemines, Gialo Oasis, Giarabub, Mechili, Merg, Saniet et-Timimi, Slonta, Soluch, Tobruch, Tocra, and Tolmeta.

For additional information on *R. s. sanguineus*, see Hoogstraal (1956A, pp. 684–724, 909–10).

OTHER *Rhipicephalus* SPECIES

R. bursa Canestrini and Fanzago 1878, was reported from Libya by Neumann (1901) but the identification was found to be incorrect by Dönitz (1905). Zanon (1923) also included this species in a list of Libyan ticks. It is questionable whether the range of this tick includes Libya.

Although *R.* (*Pterygodes*) *fulvus* (Neumann 1913) has been taken only in Tunisia, where all stages parasitize the gundi (*Ctenodactylus*) and adults attack domestic animals, it should be of considerable interest to search for this species in association with Libyan gundis. The immature stages, female, and biology of *R. fulvus* were described by Colas-Belcour (1932).

R. simus Koch 1844, reported from Misurata, Tripolitania, by Garibaldi (1935) and Stella (1938), is most probably in error.

DISCUSSION

The tick fauna of Libya has not yet been sufficiently explored to allow correlation of species distribution and ecological factors within the Kingdom.

Probably practically all of the economically important ticks of Libya are indicated in the text above. Other species remain to be discovered here; among them possibly *Ornithodoros erraticus* might prove to have an epidemiologic role. Little is known about tick-borne diseases of man and animals in Libya. Local information on *Ornithodoros* species, all suspect as vectors of relapsing fever spirochetes, is meagre. Boutonneuse fever, caused by *Rickettsia conorii*, and usually considered to be transmitted chiefly by *Rhipicephalus s. sanguineus* in northern Africa, has been reported from Tripolitania by Giordano and Nastasi (1935) and from Cyrenaica by Bartone (1950). The former authors believed that the hippoboscid fly of dogs might act as a vector during seasons when kennel ticks are not abundant on these animals. Q fever has occurred in explosive outbreaks among American troops in Tripoli (Fellers 1952A,B). The causative organism, *Rickettsia* (*Coxiella*) *burnetii*, may be harbored in indigenous or domestic animals and in their tick parasites and may eventually infect man from these sources.

Records presented above indicate that most of the North African tick species of domestic animals are now known definitely to occur within the boundaries of Libya. One of these, *Haemaphysalia punctata*, questionably included in the Libyan fauna, is a small, inconspicuous parasite that might be found on domestic animals from late autumn to early spring. Isolated populations of a few unrecorded hyalommas may be present on domestic animals in localized ecological situations.

The close, more or less obligatory, relationships between many *Hyalomma* species, wild animals, and dometic animals, shown by the data for collections from rodents, hares, and hedgehogs, is an important consideration in the planning of a control program, not only in Libya but anywhere else in the geographic range of hyalommas. Demes of *Rhipicephalus s. sanguineus* away from human habitations are equally closely associated with both wild and domestic animals in their immature and adult stages. These relationships affect the potential variety of etiologic agents that parasites can harbor or transmit and modify

epidemiological factors such as stage-to-stage and transovarial transmission of pathogens.

The known Libyan tick fauna includes three cosmopolitan species, *Argas persicus*, *Boophilus annulatus*, and *Rhipicephalus s. sanguineus*. Two species are endemic in the Mediterranean area, *Ixodes festai* of northwestern Africa and southwestern Europe, and *Ornithodoros foleyi* of North African deserts. From the Near East, *O. tholozani* and *Haemaphysalis otophila* range no further into Africa than Cyrenaica. Four hyalommas are widely distributed from the Middle East into northern Africa—*H. a. anatolicum*, *H. anatolicum excavatum*, *H. dromedarii*, and *H. impeltatum*. The last, more widely scattered in Africa than the others, occurs in drier regions as far south as Tanganyika. *Ornithodoros savignyi* has an unusual, extensive range from India and Ceylon into Africa, where it inhabits many arid and semiarid regions from the Mediterranean to southern Africa. Two hyalommas, *H. marginatum rufipes* and *H. marginatum turanicum*, have apparently been introduced from western U.S.S.R. (or nearby areas) and from tropical Africa, respectively, through the agency of migrating birds.

Tick species that obviously remain to be discovered in Libya are chiefly those parasitic on bats, tortoises, lizards, gundis, desert rodents, and hedgehogs.

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SOME HOST RELATIONSHIPS OF THE TORTOISE TICK, HYALOMMA (HYALOMMASTA) AEGYPTIUM (L.) (IXODOIDEA, IXODIDAE) IN TURKEY[1]

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ABSTRACT

Hyalomma (Hyalommasta) aegyptium nymphs, identified after having molted to adults, were found in nature in Turkey parasitizing tortoises, *Testudo gracea ibera* (their chief host); hedgehogs, *Erinaceus europaeus concolor* (a common host); an agamid lizard, *Agama stellio*; partridges, *Perdix perdix* subsp.; hares, *Lepus europaeus* (*? syriacus*); and a field mouse, *Apodemus sylvaticus tauricus*. Adults of this tick were taken from tortoises and hedgehogs. One nymph removed unfed from a hare engorged fully on a human arm and molted to an adult. All stages were feeding at various localities in July and August. By the simple technique of removing engorged nymphal ticks from hosts and holding them until they have molted to adults and can be identified to species, much valuable biological data may be obtained even under the most rigorous field conditions.

Whenever larval and nymphal ticks are collected during epidemiological field studies, almost anywhere in the world, the identity of some or all of these specimens remains uncertain owing to lack of satisfactory specific criteria for these stages. The experience described below indicates how much valuable biological data may be obtained by a very simple rearing technique even under the most rigorous field conditions.

Hyalomma (*Hyalommasta*) *aegyptium* (Linnaeus 1758) is an appellation that for half a century or more served as a "catchall" for a variety of *Hyalomma* species. Since the studies of Delpy (1946, 1949A, B) most host and geographic records under this name from Europe, Asia, and Africa have been disproven. The true *H. aegyptium* has become universally recognized as a parasite of land tortoises (*Testudo* spp.) in the Mediterranean area and Middle East. During this recent period, the belief that *H. aegyptium* parasitizes only tortoises became so definite that specialists considered all references to other hosts as suspect. It was therefore of considerable interest to us, while studying ticks on wild and domestic animals in Turkey (Hoogstraal 1959), to find immature stages of *H. aegyptium* on lizards, birds, hedgehogs, rodents, and hares, and adults on hedgehogs. Tortoises were infested by all stages of this tick.

METHODS

During 1953–1954 field trips to Turkey, tortoises and lizards were taken by hand, birds and hares were shot, and hedgehogs and rodents were live-trapped. All animals were immediately placed in stout canvas bags with tight drawstrings and carefully examined for ectoparasites as soon as possible afterwards. Engorged nymphs removed from the animals were placed in plastic tubes with plaster of paris plugs, which retained moisture, until some days after they had molted to adults, and were then preserved. Larvae, unengorged nymphs, and adults taken from hosts were immediately preserved owing to lack of facilities for rearing them in the field.

A single unengorged nymph from a hare was allowed to feed on the arm of one of us and held

[1]From Research Project NM 52 08.03.3. Bureau of Medicine and Surgery, Washington 25, D.C. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Accepted for publication September 11, 1959.

after engorgement until it had molted to an adult.

RESULTS

All adult ticks were either collected as adults or reared from nymphs. Parasite data in the lists below are noted under the host's name. Adults reared from nymphs are so indicated: LL = larvae, NN = nymphs.

Testudo gracea ibera Pallas 1827. Land tortoise. 3♂♂, 1♀, near Afyon, Afyon, July. 1N, 17♂♂, 4♀♀, Sapanca, Kocaeli, August (2 hosts). 14NN, 35♂♂, 3♀♀, Ankara, Ankara, August. 26NN; 3♂♂ reared; Tatvan, Bitlis, July. 3NN, 2♂♂, 2♀♀, near Bolu, Bolu, August.

Agama stellio (Linnaeus 1758). Agamid lizard. 1♂ reared; 4½ mi. N of Urfa, Urfa, August.

Perdix perdix subsp. Partridge. 17LL, 34NN; 2♂♂ and 2♀♀ reared; Norsinc, Bitlis, July (2 hosts).

Erinaceus europaeus concolor Martin 1838. Near Eastern Hedgehog. 20LL, 38NN; 7♂♂ reared; Norsinc, Bitlis, July. 49LL, 71NN, 2♂♂, 1♀; 6♂♂ and 10♀♀ reared; near Tatvan, Bitlis, July (5 hosts). 6LL, 33NN, 3♂♂, 3♀♀, Kara Kopru, Urfa, August.

Lepus europaeus (*?syriacus*). European Hare. 62LL, 105NN; 4♂♂ and 7♀♀ reared; Norsinc, Bitlis, July (2 hosts). 1♀ fed as nymph on human arm, Tatvan, Bitlis, July.

Apodemus sylvaticus tauricus Barrett-Hamilton 1900. Persian Field Mouse. 15LL, 4NN; 1♂ reared; near Tatvan, Bitlis, July.

DISCUSSION AND CONCLUSIONS

These data definitely indicate that immature stages of *H. aegyptium* parasitize lizards, partridges, hedgehogs, hares, and *Apodemus* mice in addition to tortoises and that some hosts are quite heavily infested. Adults feed on hedgehogs as well as their usual tortoise hosts. Lototsky (1945) also found hedgehogs, besides tortoises, to be commonly infested in Tadzhikistan.

An unfed nymph from a hare was placed on the arm of one of us (MNK) and attached readily. It fed to repletion in 3 days, dropped at night, and molted to a female several days later. Slight itching was felt at the site of attachment.

Russian workers report that adults feed from April to July, and larvae and nymphs from June to August. In Turkey, all stages were collected at elevations from between 2000 to 6000 feet in July and August.

Owing to the difficulty and uncertainty of specific identification of immature stages of *Hyalomma* ticks, practically no authoritative information on hosts of immature stages of most species in this genus has been available. Results presented above indicate how much accurate data on this subject may be obtained with simple equipment and slight expenditure of time and effort, even when in remote areas where fancier facilities are unobtainable.

Hundreds of *Hyalomma* larvae and nymphs were collected from other hosts during these trips to Turkey. Subsequent field experience has shown that if we had attempted to keep all immature ticks alive until we returned to our permanent laboratory, enough of them would have survived to provide much additional biological data after rearing to adults on laboratory hosts.

While there is no question about identity of specimens of *H. aegyptium* when collected as adults or reared to the adult stage, specific separation of larvae and nymphs of *Hyalomma* species is still so difficult that records of these stages alone are considered as only tentative. No attempt has yet been made to identify the many immature hyalommas unassociated with reared adults taken from numerous hosts in Turkey. For further details concerning *H. aegyptium*, see Hoogstraal (1956, pp. 513–516).

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THE ANATOMY AND RHYTHMICAL ACTIVITIES OF THE ALIMENTARY CANAL OF ANOPHELES LARVAE[1, 2]

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ABSTRACT

The anatomy and histology of both the mouthparts and alimentary canal and associated tissues (salivary glands and malpighian tubules) of *A. quadrimaculatus* larvae are described in detail. Physiological information on rhythmical movements found in the intact alimentary canal indicates that each major region has a high degree of activity independent of contiguous regions. The foregut and hindgut have only peristaltic contractions. The midgut exhibits predominantly antiperistaltic waves which are initiated by periodic peristaltic contractions. Effects of various drugs, selected insecticides, ligaturing, and cauterization of various ganglia suggest that most activities of the canal are essentially myogenic rather than neurogenic in character.

While the alimentary canal of mosquitoes has been intensively studied since Hooke (1665) and Swammerdam (1737)[4] made the first microscopical observations, almost no data exist on the *kinds* of movements to be found in its different regions. Although physiological studies on the motility of one or two isolated portions of the intestinal canal are available for various insects—*Aspongopus* (Nayar 1956), *Dytiscus* (Ten Cate 1924; Hobson 1928), *Periplaneta* (Yeager 1931; Griffiths and Tauber 1943a, 1943b), and *Corethra* larvae (Shaller 1949; Florey 1951; Gersch 1952, 1955; Duhr 1955; Schönfeld 1958); adult *Phormia* (Detbier and Bodenstein 1958); adult *Lucilia* and *Cynomyia* (Abbott 1945), *Apis mellifera* (Bailey 1952), and *Galleria* larvae (Beard 1958), apparently there are no detailed measurements on contraction rates for each of the major regions of the intact alimentary canal of even a single insect. Consequently we lack the most elementary quantitative physiological information on the rhythmical behavior of the insect gut as a whole.

In this paper the anatomy of the alimentary canal of *Anopheles quadrimaculatus* Say (Diptera, Culicidae) is described in detail and the different rhythmic movements (activities) found in different regions are clocked in relatively transparent, mostly quiescent, feeding, male, fourth-stage larvae. The effects of certain drugs and insecticides are briefly summarized. The effects of ligaturing and cauterization of various ganglia are also reported.

1. THE ANATOMY OF THE ALIMENTARY CANAL

Overhanging the preoral cavity (cibarial cavity, often mistakenly termed the "mouth") is a single median tuft of bristles (figure 1, mb) flanked by two large bundles of numerous fine brown hairs, the labral brushes (they are often incorrectly termed the "mouth" brushes) (figure 1, lb). When spread out the labral hairs are seen to have fine, curved tips. The brushes arise from either side of the protruding median lobe of the upper lip or labrum. When not spread out at the air-water interface, they curve downward and slightly inward. Underneath the labrum (on the upper floor of the preoral cavity itself = "epipharyngeal" apparatus of Schremmer 1949) is a complex group of fine ventral hairs which Farnsworth (1947) referred to as palatal. The median tuft of bristles and the labral brushes are actuated by two sets of muscles (figure 1, m and pm; the messorial and palatal muscles of Farnsworth 1947) which act on two flat, delicate sclerites (figure 1, ms, the messor and posterior palatal bar of Farnsworth 1947) which Snodgrass (1935) called tormae. They are best seen from the ventral side of the head.

At each side of the preoral cavity are small sharp-toothed mandibles (figure 2, md) which normally are nearly or completely obscured by an underlying pair of very prominent maxillae (figure 2, mx). Each maxilla has about 50 fine recurved hairs along the free edge. Each mandible has four conspicuous, long, pale brown, and quite flexible bristles (sickle-shaped hairs of Puri 1931 = lateral bristles and clavate hair of Shalaby 1956) which intercross at the midline (figure 2). Along the lower edges of these bristles are many extremely fine projections. Shalaby (1956) suggested that the clavate hair may be sensory. The muscles operating the mandibles and maxillae are shown in figure 2.

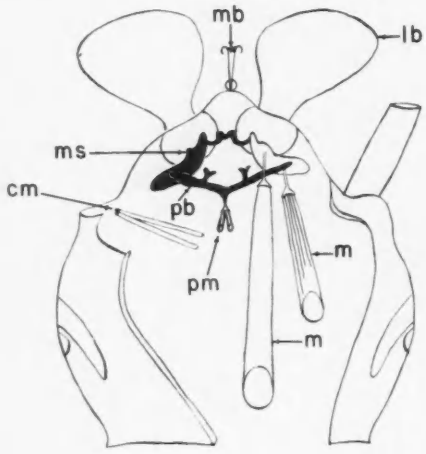
The midventral margin of the entranceway to the preoral cavity is formed by a dentate hypostomium (Snodgrass 1935, page 313, his figure 170 A), a structure which has been repeatedly and lamentably confused with the

[1]Scientific Article Number A793, Contribution Number 3061 of the Maryland Agricultural Experiment Station, Department of Entomology. Accepted for publication September 18, 1959.

[2]This paper is dedicated with affectionate esteem to Robert E. Snodgrass.

[3]The author's present address.

[4]Swammerdam made his observation on the mosquito larva in 1688.



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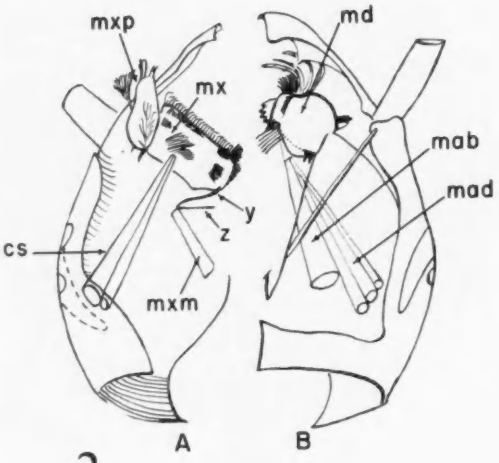

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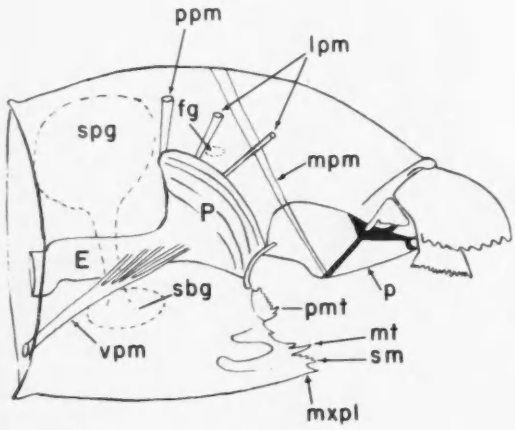

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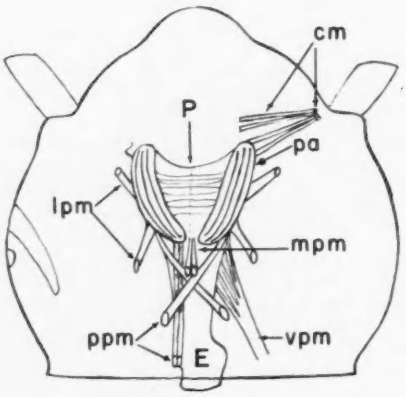


4

Schematic views of the head of fourth-stage *Anopheles quadrimaculatus* Say

FIG. 1.—Dorsal aspect of the head showing messorial muscles (m) which actuate the labral brushes (lb). Note the tormal plates: the messor (ms) and the posterior palatal bar (pb). The median palatal muscles are shown at pm, and the anterior pair of cibarial muscles are indicated at cm. The median bristles are shown at mb.

FIG. 2.—A. Ventral portion of half of the head, showing maxillae (mx), the maxillary palpus (mxp), the maxillary muscle (mxm), and the cranial flexor muscle of the stipes (cs). The sclerotized arm of the maxilla is shown at y, and the lateral rod of the prementum at z.

B. Dorsal portion of half of the head showing mandible (md) with its lateral abductor (mab) and adductor (mad) muscles.

FIG. 3.—Lateral aspect of the head to show the hypostomium (mxpl), submentum (sm), mentum (mt), prementum (pmt), pharynx (P), esophagus (E), median palatal muscle (mpm), palatum or epipharynx (p). Lateral, posterior, and ventral pharyngeal muscles are shown at lpm, ppm, and vpm, respectively. Frontal, sub- and supraesophageal ganglia are shown at fg, sbg, and spg, respectively.

FIG. 4.—A dorsal view of the head showing the pharynx (P) and its muscles. Note the pharyngeal arms (pa), the lateral (lpm), median (mpm), posterior (ppm), and ventral (vpm) pharyngeal muscles.

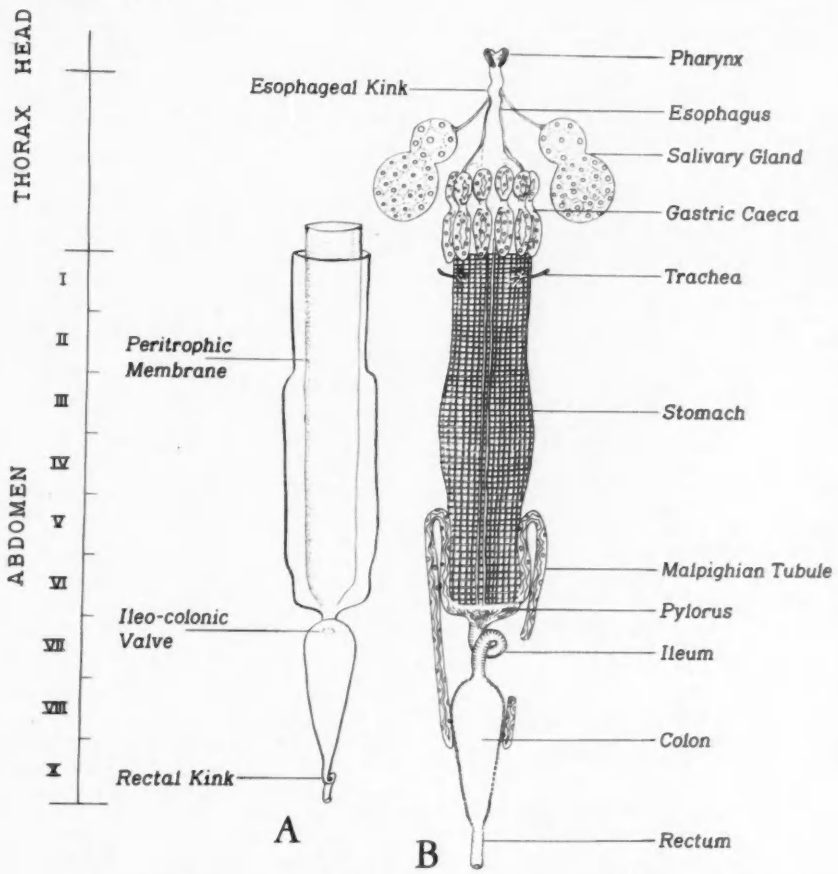


FIG. 5.—The alimentary canal of fourth-stage *Anopheles quadrimaculatus* Say larvae (schematized). A shows position of the peritrophic membrane, the swollen posterior portion of the midgut, and the position of the hindgut in the average intact larva. B shows the entire alimentary canal and associated tissues. The malpighian tubules have been partially deleted for clarity.

lower lip or labium (figure 3, mxpl)[5]. A median sagittal section through the head shows the hypostomium to be a thin outer plate which lies partially over a barbed spear-like projection which Farnsworth (1947) termed the submentum (figure 3, sm). Dorsal to the spear-like projection is another toothed spike which Farnsworth (1947) called the mentum (figure 3, mt). Between these toothed spikes of the labium and the entrance to the pharynx (the true mouth) is a complex region possessing heart-shaped flap and leaf-like structures which Farnsworth (1947) has called the prementum (figure 3, pmt) since it is ventral to the salivary gland duct opening. A tiny and inconspicuous hypopharynx lies above it. Certain imaginal mouthparts can be readily seen developing in the labium of the intact fourth-stage larva. The larval mouthparts receive nerves from the subesophageal ganglia, as Imms (1907) first showed. Shalaby (1956) did not observe a median suture in the hypostomium of *Anopheles quadrimaculatus* larvae, but it is clearly evident in our strain and in two other strains which I have examined (see also Farnsworth 1947).

According to Thompson (1905) the preoral cavity is funnel-shaped and lined with columnar epithelium. Imms (1907) surmised that the cavity contained gustatory elements: the observations of Smith (1917) and Schildmacher (1950) suggest that some such elements might actually be present, even though *Anopheles* larvae are notoriously nondiscriminating about their diet in the laboratory.

The pharynx (figures 3, 4, 5, 6, P), which is

[5]Farnsworth (1947) referred to the hypostomium as the maxillary plate, and Shalaby (1956) confusingly termed it the submentum. There are obvious advantages to calling it the hypostomium.

anterior to the supraesophageal ganglion (figure 3, spg), is a large, tilted, brown, sclerotized pouch with two conspicuous, elevated, anteriorly diverging lateral arms or crests (figures 3, 4, P and pa). The ventral pre-oral cavity and the mouth proper are clearly dilated by conspicuous dorsolateral cibarial muscles (figure 4, cm) which arise dorsally at the base of the antennae. A single median pair (figure 4, mpm) and two posterior pairs of muscles (figure 4, lpm and ppm) are inserted on the pharyngeal crests. An anterior pair of lateral pharyngeal muscles is usually quite difficult to see in living larvae, but the fanlike ventrolateral pharyngeal muscles are readily visible after the larva rotates its head (figure 4, vpm). Four intrinsic dorsal muscle bands connect the lateral arms of the pharynx (figure 4). When these bands and the posterior pair of pharyngeal muscles (figure 4, ppm) contract, they cause the pharynx to fold inward. Fine hairs (fimbriae or filtering combs) are found in the lateral arms of the pharynx as shown by Farnsworth (1947). Sautet (1935) gave an excellent description of the pharyngeal spines. According to Schremmer (1949) and Harwood (1952), when water and particles pass over the filter hairs, the large particles are retained. Secondary filter hairs, both authors suggest, push trapped material into the center of the pharynx. A cuticular intima and flattened epithelium line the pharynx. In serial cross sections the pharynx appears U- to X-shape. The pharynx and its muscles are extremely well innervated: the lateral crests conspicuously receive large nerves from both supraesophageal and frontal ganglia (figure 6).

The pharynx opens into a round muscular esophagus (figures 3E and 5) which passes through the opening between the supra- and subesophageal ganglia. In the living larva, just posterior to the brain, the relaxed esophageal tube usually can be seen to form a single well-defined lateral kink[6] (figure 5). The esophagus is lined with cuticle and has several internal folds (figure 7). Upon entering the thorax the esophagus enlarges (figure 7) and its circular muscles thicken progressively to form a gigantic, striated, annular sphincter muscle at its distal end (figures 8, 9, cm). There are some much less conspicuous striated longitudinal muscles as well (figures 8, 9, lm). The esophagus inserts into the cardia at the anterior end of the midgut proper (figures 8, 9) and thus forms a bell-shaped proventriculus or esophageal (=stomodael or cardiac) valve (figures 8, c; 9, EV) which extends down to the opening of the gastric caeca (figure 9). The invaginated portion of the esophagus, which can be easily pulled away from its connection with the stomach in dissections, maintains its rigidity when the midgut collapses. Wigglesworth (1930) commented upon this property of the proventriculus. The esophagus folds back anteriorly where it unites with the cells of the cardia (figure 9, c). There is a very abrupt change between the cuboidal cells of the esophageal valve and the columnar epithelium of the cardia. A narrow upper cap of small, tightly packed, imaginal cells is said to be interposed between the esophageal valve and the cardial epithelium (Thompson 1905). Thompson (1905), Imms (1907), and Boissezon (1930) stated that there is a blood sinus between the inner and outer folds of the esophageal valve (figure 9, BS). Schildmacher (1950) claimed that the fluid in this sinus varies according to the "activity" but he gives no experimental details. The size of the sinus apparently depends upon whether the proventriculus is relaxed or contracted (see Wigglesworth 1930, his figure 1).

According to Thompson (1905) "delicate longitudinal fibers from [the outer wall of] the esophagus cross to the shoulder of the cardia outside the valve . . .". Imms (1907) also observed this "fibrous band" and said he believed it ". . . to be derived from the outer connective tissue coat of the esophagus." While the kind of fibers that Thompson (1905) and Imms (1907) illustrated along the cardia were not seen in this study, two thin lateral bands of nonfibrous tissue are visible passing down the esophagus (figures 8, 9, cg) and extending along the lateral edges of the cardia. Possibly some of the tissue is truly connective (or even glandular), but there are also present within a band two nerve cells in tandem: the anterior one sends out two side branches of equal size to the lower esophagus, and the more posterior neuron sends out one large and one much smaller nerve to the region of the cardia and caeca (figures 8, 9, cg). Presumably these cells constitute the stomachic (ventricular or postesophageal ganglion) ganglion.[7] This band of tissue is not mentioned by recent investigators (e.g. Schildmacher 1950). Kuwana (1935) found both circular and longitudinal esophageal muscles of the silkworm were innervated. Other possible sites of gut innervation were not intensively studied in the *Anopheles* mosquito larva; even so, it may be said that no other obvious nerve fibers or conspicuous ganglia were seen in this study on other portions of the fore- and midgut of the alimentary canal.

In the hemocoel at each side of the cardia lie two large salivary glands (figures 5, 7, sg).

[6]Salem (1931) is the only author I have found who mentioned the presence of this kink, which he called a cul-de-sac.

[7]Sharif (1937), who gave a good description of the innervation of the mouth-parts and alimentary canal in the rat-flea larva, said that nerves from the stomachic ganglion are distributed on the mesenteron. Day and Powning (1949) found striking differences in innervation of the alimentary canal in various Orthoptera. Puri (1925) gave an excellent description of the stomatogastric nervous system in *Simulium*.

Each is composed of two globular sacs, a small anterior one having few cells with large nuclei and a larger posterior sac with smaller nuclei (Jensen and Jones 1957). The glands empty into ducts devoid of taenidia.[8] The ducts unite into a common tube within the head. Imaginal salivary glands arise from a narrow band of very tightly packed cells encircling the uppermost end of the anterior sac. A thin ventral chain of syncytial cells extending between the two glands in the prothorax takes up ammonia carmine and has been termed the ventral nephrocyte chain (Métalnikov 1902).

The midgut or ventriculus or stomach begins with the cardial cells (figure 9, c) which according to Imms (1907) secrete the peritrophic membrane. The view shown in figure 9 suggests the possibility that the small cells at the upper end of the outer esophageal fold are more active in the formation of the peritrophic membrane than the larger, dark-staining, columnar epithelium of the cardia proper. The cardial cells have a distinct brush border facing the gut lumen, and small, darker staining, regenerative cells fit into their base either singly or in groups.

Posterior to the cardia, the midgut is encircled by eight[9] large, evaginated, finger-like pouches termed the gastric caeca (figure 5). In *Anopheles* larvae, these diverticula of the midgut are constricted into small anterior and large posterior sections. Salem (1931) said there are no constrictions in the caeca of *Aedes*. They extend from the prothorax to the end of the metathorax. The caecal cells are quite large, flattened, polygonal, and have a well-developed inner brush border. The cells have granular cytoplasm. In partially crushed whole mounts the cells may be disrupted in such a way that they round up into spheres and the filaments of the brush spread out around them in discrete units somewhat resembling cilia. Thompson (1905), Imms (1907), Boissezon (1930), and Samtleben (1929), did not find intrinsic muscles on the caeca. Raschke (1887) briefly stated that the caeca of *Aedes* larva have a muscular tunic. Schildmacher (1950) contended that after silver staining he observed delicate ring muscles on the caeca, but he did not give their location or distribution. In the material examined in this study the caeca appeared devoid of muscles[10]. Puri (1925) found circular caecal muscles in *Simulium* larvae.

The midgut from the opening of the gastric caeca to the sixth abdominal segment is a straight tube (figure 5) called the stomach (Imms 1907). In the living, intact *Anopheles* larva the stomach can often be seen to be divided into a somewhat narrow anterior zone (usually in the first two abdominal segments) and a wider posterior zone (figure 5, A). Usually only a central zone—between the third and fourth or fifth abdominal segments—is enlarged[11] as diagrammed in figure 5B. The food column is uniform in diameter throughout the midgut. The stomach in intact larvae tends to narrow as it approaches the hindgut, but this is not evident in freshly excised preparations. Bruck's (1930) statement that the stomach of *Anopheles maculipennis* larvae is devoid of muscles was in error, for it is invested by an extensive lattice-like arrangement of outer longitudinal and much smaller, inner, circular muscles (figure 5B) as Raschke (1887), Thompson (1905), Imms (1907), Boissezon (1930), and many others have demonstrated. In *A. quadrimaculatus* larvae there are two very conspicuous outer dorsal longitudinal muscles running the length of the midgut. Imms (1907) and Samtleben (1929) asserted that the midgut muscles are striated, but in fresh, unstained, whole gut mounts of *Anopheles* larvae the striations are very indistinct.

While observations on whole mounts and sections of the stomach did not reveal any very marked *structural* differences between the cells in the anterior and posterior halves,[12] the work of Boissezon (1930) and Wigglesworth (1942) indicated there are histochemical differences. Bowen's (1951) work on *Drosophila* larva indicated that material outside the peritrophic membrane moved more slowly than inside it. My findings with fourth-stage *Anopheles* larvae do not agree with those of either Federici (1922), Samtleben (1929), or Richins (1945) when they stated that the anterior midgut cells lack a brush border or that the anterior cells are larger than the posterior ones.[13] In disagreement with Schildmacher (1950), the cells in the middle region of the *Anopheles* stomach possess a brush border.[14] Possibly these differences arose from using larvae of widely different ages or of varied nutritional states, or from dissimilar techniques of preparing the gut for examination, or to species or staining differences. Verolini (1949), on the base of histology, claimed that the anterior midgut absorbs water and that the posterior part is secretory in *Culex* larvae.

The midgut cells vary considerably in size even

[8]Raschke (1887) also failed to see taenidial rings in the salivary duct of the "*Culex nemorosus*" (=*Aedes*) larvae.

[9]Wigglesworth (1949) incorrectly stated that there are five gastric caeca in mosquito larvae (p. 286). Neveu-Lemaire (1938) incorrectly showed no gastric caeca in *Theobaldia* larvae (see Sautet 1935).

[10]According to Richards (personal communication), the gastric caeca of *Aedes aegypti* have very fine (barely resolvable) birefringent fibers in addition to the connective tissue sheath and plasmal membranes.

[11]Gersch (1952) observed enlargement of the middle portion of the stomach in *Corethra* larvae.

[12]In the rat-flea larva regenerative cells are more numerous in the anterior half of the midgut (Sharif 1937). According to Richins (1945), the same is true for *Aedes* larvae.

[13]Cox (1938) claimed that during secretion the midgut epithelium of the blowfly does not show a striated border.

[14]Boissezon (1930), on the basis of his studies, stated that the height of the brush border bears a relation to cellular activity.

in the same general region as shown by stained whole mounts (figure 11). In such flattened preparations the stomach cells appear ovoid to polygonal, and the smaller regenerative cells are almost triangular (figures 10, Rc, 11). In sections the stomach cells vary from cuboidal to columnar and, except for regenerative cells, possess a distinct brush border of variable height. The basement membrane is visible in whole mounts and in sections.

The scattered small "nerve cells" which Schildmacher (1950, p. 399) stated he found in a single (?) preparation innervating the circular muscles of the middle and posterior regions of the midgut in mosquito larvae,[15] were not visible in *Anopheles quadrimaculatus* whole mounts. Nerves to the midgut are often curiously difficult to stain (see Kuwana 1935). Tracheal end-cells, sessile hemocytes, and connective tissue cells can take up methylene blue as well as silver, and may then closely resemble nerve cells or their fibers.[16]

The midgut receives a segmental tracheal supply from lateral branches of the dorsal longitudinal tracheal trunks. The tracheae appear to serve to suspend the gut in the hemocoel.

Five round, thin, elongated malpighian tubules (figure 5) empty around the posteriormost end of the midgut above the pyloric chamber. They are devoid of muscles. These tubes pass upward alongside the midgut for only a short distance, usually to the fifth or sixth abdominal segment, before becoming folded posteriorly. Some of the tubules cross each other in the seventh abdominal segment before becoming finally aligned alongside the colon. Their blind ends occur mostly in the seventh and eighth abdominal segments; they never extend into the tenth or anal segment. The tubules are composed of large, flat, curved, polygonal cells (figure 12) that have a large nucleus and a well defined brush border. The tubules are held in position by connective tissue filaments and tracheae. During a stadium the cytoplasm becomes densely filled with inclusions[17].

Midgut epithelium in *Anopheles* larvae abruptly ends before the pyloric chamber. The pylorus is a very thin-walled, transparent funnel of greatly flattened epithelium which narrows sharply into a long, round, densely muscular ileum. The ileum is situated between the sixth and seventh abdominal segments of the intact larva. If the ileum is torn away from the midgut, it can be seen that the malpighian tubules empty above and into the pyloric chamber (figure 5B), which often remains attached to the midgut. There is truly a sphincter at the base of the pylorus (the pyloric valve); sometimes it can be seen unmistakably contracting in favorable, intact larvae and in some freshly isolated guts. Thompson (1905) did not see a valve separating the ileum from the stomach in *Culex* larvae (it is difficult to see in *Anopheles* unless it contracts), but Boissezon (1930) figured it in his study. At the upper end of the ileum in *Anopheles* larvae are four rows of numerous sharp, caudally-pointed spines arranged in scalloped rings as shown in figure 13. Trembley (1951) gave a good description of these spines in various adult mosquitoes. The ileum with its numerous powerful circular muscles coils once upwards to open into a capacious elongated colon (figure 5B).

In the intact *Anopheles* larva the colon is seen as a tapering bulb in the seventh to eighth abdominal segments. When it contracts it folds into a compact, tightly narrowed tube. It is composed of very large flattened, polygonal or ovoid cells densely overlaid with numerous very large, regularly spaced, striated, circular muscles (figure 14, cm) and with fewer broad, flattened bands of longitudinal muscles (figure 14, lm). The colon is the most densely tracheated region of the alimentary canal. The horizontal colon turns ventrally and opens into a short, thin-walled rectum (figure 5B) of flattened epithelium. The rectum occupies the tenth (anal) segment. In the intact *Anopheles* larva, the relaxed rectum forms a kink where it joins the colon (figure 5A). When the rectum discharges its contents, the anal papillae spread out.

2. FEEDING BEHAVIOR

Anopheles larvae are able to feed without rotation of the head when they eat filaments of algae (Smith 1917) and when they graze off the bottom or sides of shallow containers (Puri 1931; Jones 1954). In the most characteristic method of feeding, the head swivels through a 180° angle[18] (Nuttall and Shipley 1901), and larvae usually feed quietly in one place. Their large, cup-like preoral cavity breaks the water surface at a slight angle, as described by Christophers and Puri (1927) and Renn (1941). Renn (1941) wrote, ". . . the maxillary palps, the maxillae, and the submentum [form] . . . a raised, V-shaped barrier along the posterior rim of the mouth [and they] are unwetted and protrude through the (air/water) interface." The brown labral brushes, which are wetted and so do not break the air-water interface, usually are thrust in downward arcs with great rapidity[19] and with what appears

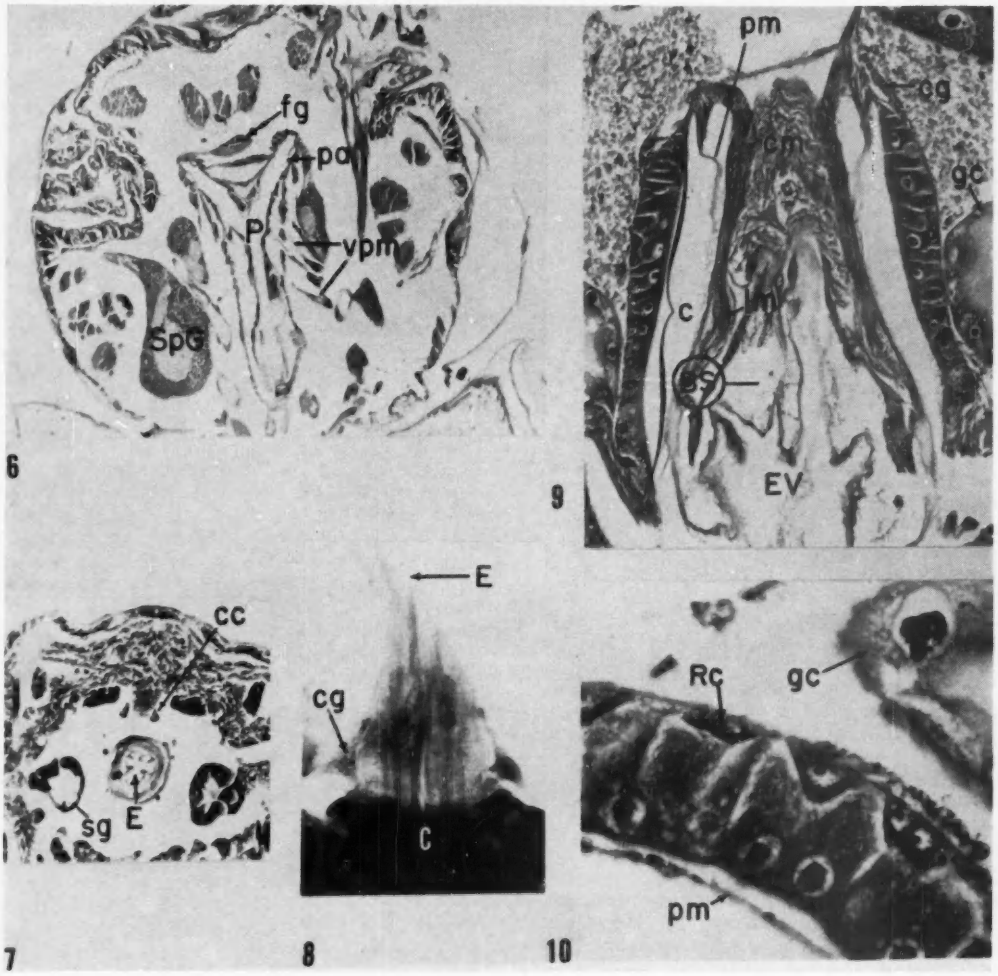
[15]Plotnikova (1949) contended that the unpaired nerve in the abdomen of the *Aeschna* larva sends branches which innervate the dorsal and ventral surfaces of the intestinal canal. Her various findings suggested that one neuron innervates both somatic and intestinal musculature.

[16]Schönfield (1958) has recently illustrated nerve cells on the alimentary canal of *Corethra* larvae.

[17]Tulloch and Goldman (1942) gave a useful description of Malpighian tubules of *Aedes aegypti*.

[18]Some other larvae of Nematocera rotate their heads during feeding, e.g. *Simulium* (Puri 1925).

[19]According to Bekman (1936), the labral brushes of *Anopheles maculipennis messeae* make more than three strokes per second.



FIGURES 6 THROUGH 10

Photomicrographs of various portions of the alimentary canal of *Anopheles quadrimaculatus* Say larvae

FIG. 6.—Horizontal section through head showing frontal ganglion (fg), pharynx (P), with its lateral arms (pa), ventral pharyngeal muscles (vpm), and portion of supraesophageal ganglion (Spg).

FIG. 7.—Cross-section through the prothoracic region showing esophagus (E); note circular musculature and folded intima. The anterior sacs of the salivary gland are indicated at sg. The corpora allata-cardiaca complex is shown above the foregut at cc.

FIG. 8.—Whole mount of esophagus (E), lateral strand of tissue (cg) containing the nerves which innervate the lower esophagus and the upper cardial region (C).

FIG. 9.—Horizontal section through esophageal valve (EV). Note cardial cells (c), giant circular muscle (cm), longitudinal muscles (lm), lateral mass of tissue (stomachic ganglion) (cg), peritrophic membrane (pm), gastric caecum (gc), and blood sinus (BS).

FIG. 10.—Cross section through anterior midgut to show peritrophic membrane (pm), regenerative (imaginal) gut cells (Rc), and a portion of a gastric caecum (gc). A delicate brush border can just be made out on the free border of the midgut cells.

perfect synchrony where food particles are small (or when they are entirely or largely absent). When *Anopheles* larvae are about to stop feeding or when food particles are large, the labral brushes contract more slowly and often move asynchronously.

Food is brought towards the mouth either by eddy-feeding or film-feeding (Renn 1941). Small particles are swept directly into the pharynx but particles larger than the mouth opening must be rasped or crushed into suitable size by the mandibles. The mandibles move horizontally but they are incapable of crushing small particles, since the teeth do not mesh at the midline. According to Puri (1931) the mandibles are kept closed while the labral brushes are working, but this is not true for *A. quadrimaculatus*. The maxillae may briefly clasp larger food masses. *Anopheles* clearly do not prefer to eat large particles and either bounce them away from the mouth before they are seized, or turn the head slightly and thrust the food particle to one side when the mandibles and/or maxillae have already engaged it.

Every 30 to 40 seconds the fanned-out labral brushes are folded down into the preoral cavity as tightly narrowed, blade-like bundles. Sometimes, as the lowered brushes are raised again into the sweeping position, the antennae are momentarily deflected towards the midline. If water is withdrawn from around *Anopheles* larvae, they cannot lift the labral brushes. Insertion of the brushes into the preoral cavity (into the mouth itself?) occurs whether food is being taken in or not, and so is not specifically associated with packing food into the pharynx.

One-hour-old fourth-instars of *A. quadrimaculatus* spend about one-half of their time in feeding, whereas 24- to 72-hour-old larvae appear to feed almost continuously (table 1). Hurst (1890) claimed that mosquito larvae near pupation were unable to eat.

The midgut in the living *Anopheles* larva varies in natural color from very pale yellow to a deep golden yellow. The food column (in larvae fed yeast and dog food) usually is dark brown. When the gut contents are removed by dissection the foregut and midgut appear mostly colorless, as does the ileum, but the colon retains its brownish cast. These natural colors of the gut interfere with colorimetric pH determinations on intact larvae. The caeca frequently contain particulate matter. Phenol red[20] stains the caecal contents pale pink to orange or yellow; the food column appears bright red down to the fourth or fifth abdominal segment, where it usually becomes orange. There is a definite concentration gradient in *A. quadrimaculatus*, for the anterior end of the stomach stains a darker red, and the color gradually lightens posteriorly[21]. Food passing into the hindgut stains pink or red. The egesta are red too. If the *Anopheles* larva is fasted and then offered food, the feces are at first pale red in the dye solution but soon become brilliant red as the larvae ingest food particles. Thus, the alimentary canal of *A. quadrimaculatus* is largely alkaline in reaction but possesses slightly acid to neutral caeca. Food particles did not stain in the pharynx and esophagus.[22] It is suspected that the salivary gland secretions are alkaline because a faint red spot can be seen just in front of feeding larvae in phenol red.

3. RHYTHMIC ACTIVITIES OF THE ALIMENTARY CANAL OF *Anopheles quadrimaculatus* LARVAE

Even in the absence of food, the pharynx of *A. quadrimaculatus* is usually held in a state of considerable tension by the posterior pair of cibarial muscles which twitch more or less continuously, causing the pharyngeal arms to quiver (figure 15, a). When abundant food is available, it is drawn into the pharynx where it generally gathers into a definite bolus conforming approximately to the size of the pharynx. The pharynx contracts (figure 15, b) and the bolus enters the esophagus. The esophagus constricts and a small peristaltic or catastaltic wave (hereafter referred to as the p-wave) carries the bolus swiftly to the midgut (figure 15, b, c). Then the esophagus relaxes (figure 15, a). However, contractions of the pharynx do not necessarily induce esophageal contractions. P-waves of the esophagus in *Anopheles* larvae occur every 1 to 30 seconds independently of food intake (table 1). This rate of swallowing is in agreement with the value given by Purdy (1920). It is not in agreement with Schildmacher (1950), who stated (p. 419) that esophageal contractions are rare in *Anopheles* larvae in the absence of food. Esophageal p-waves tend to be more frequent, yet more irregularly spaced, during actual ingestion of food.[23] Small to large boluses may be held 15 to 30 seconds in the esophagus at the kink before being swept posteriorwards: thus, the presence of the bolus, even when large, does not initiate additional p-waves.[24] While the esophagus is capable of small antiperistaltic waves, they almost never occur in normal larvae. The salivary glands do not move.

The behavior of the cardiac sphincter could not be observed in intact or in freshly excised preparations from *Anopheles* larvae. However, in

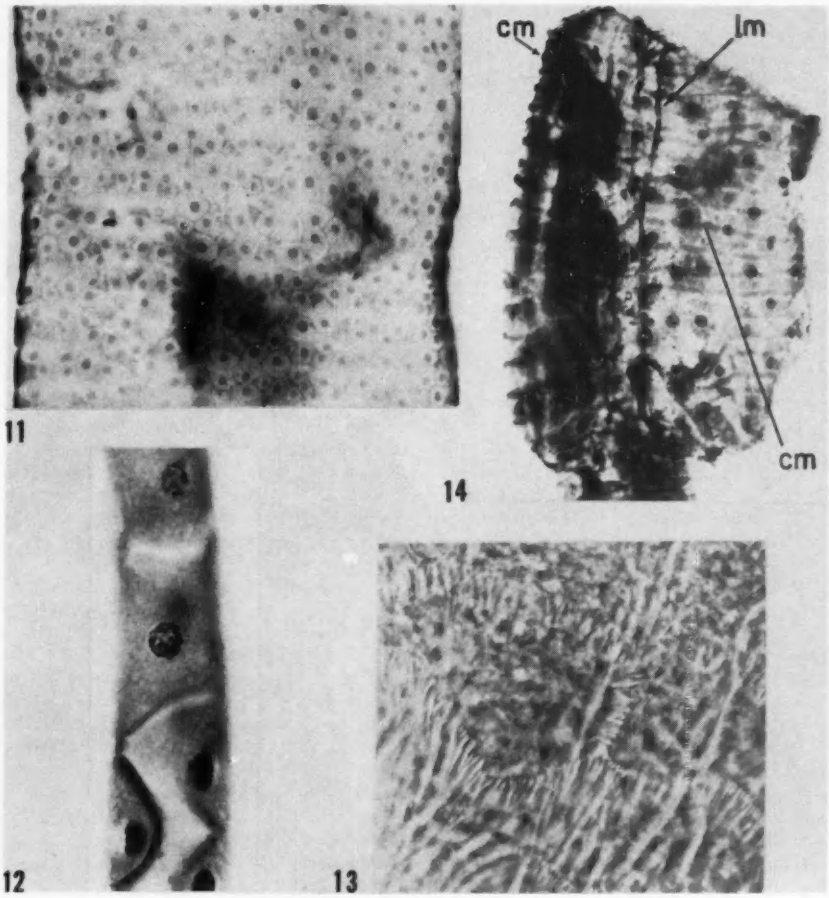
[20]A variety of other indicator dyes were also used, but phenol red seemed to give the best results.

[21]This is not in agreement with Senior-White (1926), for he stated that alkalinity increased towards the posterior end of the midgut (p. 228).

[22]According to Gersch (1952), the pharynx of *Corethra* has no secretory cells.

[23]Bruck's work (1930) indicated that the rate of passage of food in *Anopheles* larvae depended on the intensity of food ingestion.

[24]In *Corethra*, Gersch (1952) found that ingestion of food stimulated midgut peristalsis.



FIGURES 11 THROUGH 14

Photomicrographs of certain portions of the alimentary canal of *Anopheles quadrimaculatus* Say larvae

FIG. 11.—Whole mount of a portion of the larval stomach with peritrophic membrane and food column mostly removed. Note variations in size of the larval cells and the smaller, darker-staining, triangular regenerative cells.

FIG. 12.—A portion of a whole mount of a malpighian tubule.

FIG. 13.—Pyloric spines in an unstained whole mount.

FIG. 14.—Whole mount of colon showing circular muscles (cm) and longitudinal muscles (lm).

excised gut preparations from *Aedes aegypti* larvae the sphincter readily contracts. Day and Powning (1949) referred to the writhing movements of this region in roaches. Wigglesworth (1930) observed that when the blood sinuses of the proventriculus in *Culex* and *Aedes* were distended, an occlusion of the lower end of the esophagus resulted and he stated that this closure of the orifice served to drive onwards the contents of the gut (p. 598). Contrary to Wigglesworth's (1930) suggestion, the proventriculus in *Anopheles* larvae definitely functions as a valve since it permits the one-way passage of food through an orifice and is apparently very effective in preventing regurgitation.[25] Boissezon

[25] *Corethra* normally regurgitate food from the stomach into the pharynx, according to Gersch (1952). But in this larva peristaltic and antiperistaltic waves are also normal in the esophagus. In *Corethra*, then, the proventriculus does not function as a valve.

(1930) and Schildmacher (1950) both remarked that this valve prevented regurgitation. Wigglesworth (1930) believed the proventriculus acted as a sphincter for milling the peritrophic membrane, but based this conclusion on its position and from histological preparations showing the annular muscle of mosquito larvae in various states of contraction or relaxation. Duhr (1955) subsequently observed rhythmic contractions of the proventriculus ("valvular gland") in intact *Corethra* larvae and claimed that he observed secretion of the peritrophic membrane.

Different portions of the midgut vary in their rhythmical activities. The four pairs of gastric caeca move with vigorous pumping thrusts but never synchronously.[26] The movement is a definite antiperistaltic or anastaltic wave (hereafter termed ap-wave) (figure 16, a, b). In some cases the movement begins where the large and small caeca join to empty into the midgut. Schildmacher (1950) observed caecal contractions but spoke of peristaltic waves. Their marked,asynchronous, piston-like bchavior in *Anopheles* larvae is very difficult to account for, first, because the caeca appear to lack any sign of muscle, and, second, because their activity is independent of contractions of both esophagus and stomach proper, as will be seen later. Caecal contractions in *A. quadrimaculatus* are more or less continuous at a rate of about 60 per minute, regardless of actual ingestion of food. Pouchet (1847) stated that the caeca of *Culex pipiens* larvae contract every 25 to 30 seconds.

A series of generally large, relatively slow waves move anteriorly over the lower half of the stomach (figure 16, d) at a rate of about 70 per minute. On reaching the middle part of the stomach (abdominal segments 3 and 4) they abruptly change into a series of usually smaller (figure 16, d) and often much faster antiperistaltic waves at a rate of about 105 per minute.[27] These waves ripple for variable periods depending upon the age of the larvae (table 1) and then fade out and the whole stomach ceases moving (figure 16, a), although normally the caeca continue contracting. The midgut then enlarges (relaxes?) (figure 16, b), especially in its central portion, and appears to jerk forward; shortly after this a single, very large, and powerful p-wave appears on the midgut[28] (figure 16, c) and reinitiates the smaller ap-waves (figure 16, d). The single p-wave originates either in the first or in the third abdominal segment. In the former case the catastaltic (p) wave moves down to about the second to fourth abdominal segment. When the p-wave originates in the middle of the stomach (abdominal segments 3 and 4), it quickly spreads out both anteriorly and posteriorly. Immediately following this great contraction the newly induced ap-waves are distinctly larger and more rapid. Within a short time, however, the ap-waves have a lowered amplitude and move at a reduced rate. As shown in table 1, the large p-contractions of the stomach of *Anopheles* larvae appear to decrease with age in the fourth stage. The rhythmical activities of the stomach constitute a three-phase gastric cycle: (1) anastaltic phase, (2) quiescent phase, and (3) catastaltic phase. This cyclic activity apparently is not related to the relatively slow backward passage of the food column.[29]

The midgut of *Anopheles* larvae tends to shift anteriorly while the ap-waves are in progress; the resulting tension of the alimentary canal produced by these ill-defined shifts appears to be related to the contractions of the ileum. P-waves of the ileum may average 33 per minute (table 1). Contractions of the ileum can occur independently of contractions of the pyloric sphincter. Malpighian tubules have no intrinsic motility[30]

Vigorous peristalses of the ileum do not induce contractions of the colon. When the colon contracts, its anterior end trembles, contracts and expands somewhat like a swaying bellows, several partial contractions follow each other rapidly, and then the whole tube suddenly constricts with great force and thrusts very sharply backwards. In most cases the egesta are not visibly enclosed by portions of the colorless peritrophic membrane. Imms (1907) said that the peritrophic membrane is shed at the molt through the anus. Contractions of the colon may be sufficiently strong to lead to brief but often extensive protrusion of the rectum.

4. SOME PRELIMINARY EXPERIMENTS ON THE NATURE OF THE RHYTHMICAL ACTIVITY OF THE ALIMENTARY CANAL OF *Anopheles quadrimaculatus*

Both ingestion and egestion rates as measured by the phenol red technique (5 to 10 larvae placed in 25 ml. of .005% phenol red under feeding and fasting conditions) are highly variable even in very uniform-looking *A. quadrimaculatus* larvae reared under highly controlled conditions. In some experiments larvae were kept in trypan blue before placing in phenol red. It was tentatively concluded from several hundred tests that (1) when larvae are transferred from one dye to different dye solutions, the dyes do not form distinct layers but become mixed. Schildmacher (1950) (p. 414) stated first that sharp layering

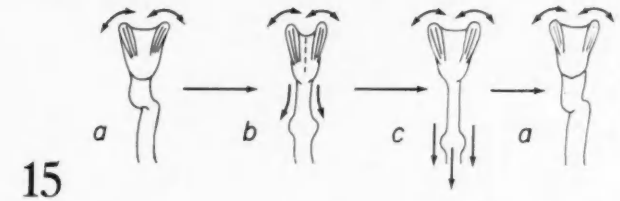
[26]Sautet (1935) briefly noted "incessant" contractions of the caeca of mosquito larvae.

[27]Yeager (1931) observed antiperistaltic waves of different speeds on the crop of the cockroach.

[28]In *Corethra* the p-wave apparently is induced by ingestion of food alone (Gersch 1952).

[29]Bruck (1930) stated that the midgut of *Anopheles maculipennis* larvae was devoid of peristaltic movements.

[30]According to Keilin (1921), the malpighian tubules in many dipterous larvae are devoid of peristaltic movement.



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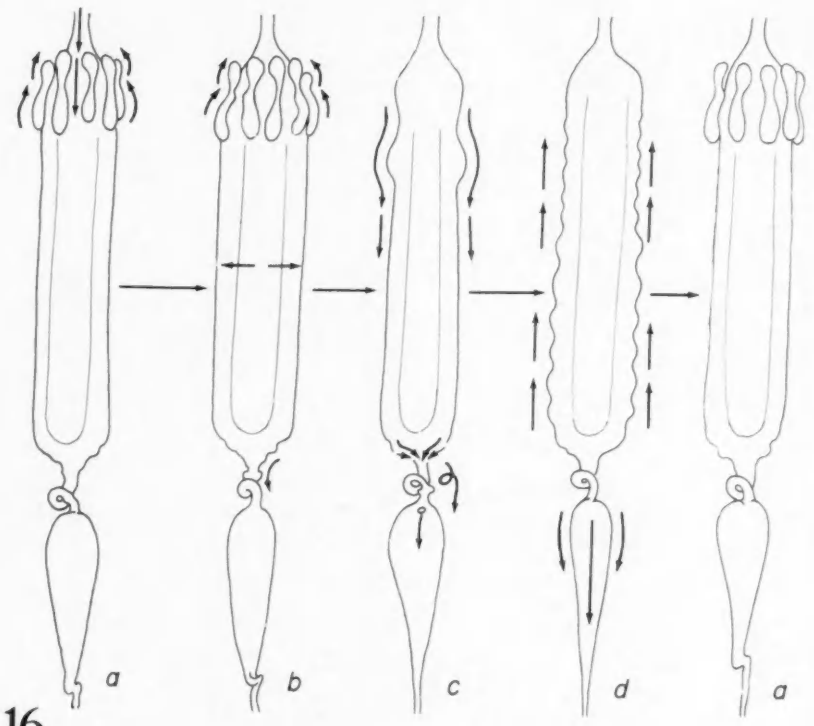

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FIGURES 15 AND 16

Movements of the alimentary canal of *Anopheles quadrimaculatus* Say (diagrammatic)

FIG. 15.—*In vivo* contractions of pharynx and esophagus. a. Quivering of pharyngeal arms. b. Contraction of the pharynx and beginning of the esophageal peristaltic wave; the dotted vertical line in the pharynx indicates this region folds inward. c. Passage of the esophageal wave, quivering of the pharyngeal arms; note kink appears on the relaxed esophagus (a).

FIG. 16.—*In vivo* movements of midgut and hindgut. a. Food is brought into the midgut through the esophageal valve, the gastric caeca pump whether the midgut is active or momentarily inactive; the midgut in a. has just ceased moving. b. The midgut appears to enlarge; contractions of the ileum may begin and are always posteriorly directed. c. Single, large-wave contraction passes posteriorly down the midgut; the pyloric valve may contract and the ileum carry material into the colon. d. Antiperistaltic waves sweep upward over the midgut following the peristaltic wave; the colon may contract and the rectal kink then straightens out. The malpighian tubules have not been shown in any of the figures. The gastric caeca are not shown in figures c and d. The food column is indicated in the midgut in each figure.

Table 1.—Rates (in seconds) of rhythmical activities of different regions of the digestive tract of fourth-stage *Anopheles quadrimaculatus* larvae at 27 -28° C. Mean values are in parentheses.

Type of Activity	Age of larvae when data were recorded					
	1 hr.	19–20 hrs.	24 hrs.	45–48 hrs.	48 hrs.	72 hrs.
Feeding time in 1 minute	0 –60 (33.0)	0 – 60 A	28 –60 (52.2)	4 – 60 (45.2)	50 – 60 (57.7)	30 – 60 (58.0)
Time between single esophageal waves	1.2–14.8 (6.2)	2.8– 28.8 (8.8)	4.4–17.8 (9.3)	3.8– 20.0 (8.1)	3.0– 26.8 (7.2)	2.4– 19.6 (7.3)
Time for 10 caecal movements	5.8–12.0 (8.2)	6.8– 9.4 (8.1)	8.4–15.6 (11.8)	8.4– 13.4 (9.8)	7.4– 13.0 (10.2)	7.6– 11.6 (9.1)
Time for 10 ap-anterior midgut waves	3.4– 6.2 (4.5)	4.2– 11.2 (6.8)	3.2–13.4 A	4.2– 10.8 (8.0)	3.4– 9.2 (5.0)	B
Time for 10 ap-posterior midgut waves	4.4–10.6 (8.0)	B	6.6–33.0 (9.4)	C	3.4– 14.8 (8.0)	4.4– 10.6 (8.5)
Time between single large p-contractions over midgut	25.0–63.2 (44.3)	35.0–125.8 (69.0)	15.0–94.0 (57.7)	74.4–126.2 (95.2)	75.0–117.0 (101.6)	29.0–189.0 (107.4)
Time between single ileal contractions	C	C	C	C	9.0– 56.0 (18.7)	15.0– 29.0 (18.3)

A: Data insufficient for averaging.
B: Area obscured so that readings could not be made.
C: No observations made.

occurred, but later (p. 421) contended that mixing of the midgut contents occurred and was due to midgut contractions. In *A. quadrimaculatus* the food column is not visibly deformed during contractions of the midgut: the column appears to be held uniform within the stiff, cellophane-like peritrophic membrane. (2) More phenol red enters the gut and enters it more rapidly if food is present (100% cases positive in 20 minutes) than if food is not available (50% cases positive in 45 minutes).[31] (3) The gut clears of phenol red more rapidly when larvae are feeding (e.g., 90% cases cleared the dye from the gut in 4 hours) than when larvae are fasting (40% cases clear in 4 hours). (4) Defecation rates are, however, *insignificantly* altered if freshly fasting larvae are given food, or if feeding larvae are freshly fasted. Rates do not significantly vary during an hour of feeding nor during an hour of fasting. (5) Atropine sulfate (0.1%), fresh adrenaline hydrochloride suspensions (approximately 0.01%), and digitalis leaf suspensions (approximately 0.1%) had no definite effects on the defecation rate[32] (this agrees with the findings of Ten Cate 1924), but nicotine (0.01%) and pilocarpine[33] (0.1%) seemed to increase defecation considerably over either fasting or feeding controls. (6) Toxaphene (10^{-3}), phenothiazine (10^{-5}), DDT[34] (10^{-5}), and parathion[35] (10^{-6}) in lethal doses did not greatly alter the rate of defecation prior to the moribund stage.

When the alimentary canal of an *Anopheles* larva is excised in a drop of buffered *Drosophila* saline (Ephrussi and Beadle 1936) the rhythmical activities of the midgut immediately and completely disappear.[36] The activities of the fore- and hindgut are also seriously depressed though there is a definite tendency for the hindgut to retain more activity than the foregut. This rapid loss of rhythmical activity is in striking contrast to the vigorous, sustained, and elaborately coordinated activities of the excised imaginal gut in the same saline. To study why the excised alimentary canal of the larva should lose most of its activity, intact larvae were ligatured or cauterized in various ways. Black surgical silk ligatures (Deknatel, type B, 6–0) were tied slowly, firmly, and tightly between various body segments; often somewhat more than the desired intersegmental region was unavoidably included. Various ventral ganglia of unanesthetized larvae were cauterized under a dissecting microscope with an extremely fine wire loop fitting into a special cauterizing outfit modified after Howland and Robertson (1934), or with a Storz cauterizing outfit (Storz Instrument Co-Company, St. Louis, Mo., catalogue item No.

[31]According to Bruck (1930), food passes through the alimentary canal of *Anopheles maculipennis* larvae in 40 to 60 minutes. Purdy (1920) gave the passage time as 30 to 45 minutes.

[32]Such data are of limited significance, for there is no assurance that the drugs entered the larvae or reached critical sites in effective concentrations. In *Daphnia* atropine is said to abolish effects of acetylcholine (Obreshkove 1941).

[33]Voskresenskaya (1936) stated that atropine, pilocarpine, and adrenalin reduced the contractions of the alimentary canal of lepidopterous larvae (*Euxoa* and *Porthetria*).

[34]According to Florey (1951), DDT increases the activity of the intestine in "arthropods," i.e., in *Corethra*.

[35]Kooistra (1949) found that parathion stopped movements of excised gut in *Periplaneta*.

[36]I have only recently discovered that most portions of the excised alimentary canal of *Aedes aegypti* larvae retain considerable activity. Schildmacher (1950) stated that excised guts of eight species of mosquito larvae in "Ringer" solution showed peristalsis, but he did not designate which areas contracted.

Table 2.—Effect of ligaturing on the rhythmic activities of the alimentary canal of intact, 24-hour-old, male fourth-stage *Anopheles quadrimaculatus* larvae. Observations varied from 5 to 70 minutes per larva (average 15–40 minutes); 27°–28° C.

Ligature site	No. larvae	Percentage with rhythmical activities in[1]												Mean activity of alimentary canal
		Mb	P	E	GC	1	2	3	4	5	6	II	C	
Head-thorax	3	——	——	—[2]	100.0	66.7	33.3	33.3	33.3	33.3	33.3	100.0	100.0	59.2
Thorax-I AS[3]	6	66.6	100.0	80.0	20.0	—[2]	83.3	100.3	66.7	33.3	33.3	100.0	100.0	71.2
I-II AS	11	36.4	90.9	45.4	36.4	——	——	45.4	36.4	9.1	18.1	100.0	45.4	46.4
II-III AS	8	75.0	75.0	50.0	62.5	12.5	——	——	62.5	62.5	25.0	87.5	50.0	56.3
III-IV AS	11	58.3	83.3	70.0	25.0	0	0	——	——	33.3	33.3	66.7	72.7	44.3
IV-V AS	9	66.6	88.8	66.6	55.5	0	0	0	——	——	22.2	44.4	50.0	39.4
V-VI AS	7	50.0	100.0	57.1	71.4	0	28.5	28.5	42.8	——	——	14.2	42.8	43.5
VI-VII AS	6	50.0	83.3	50.0	33.3	33.3	16.6	50.0	16.6	0	——	——	33.3	36.6
VII-VIII AS	7	71.4	71.4	42.8	28.5	14.2	28.5	42.8	28.5	0	28.5	——	——	35.7
VIII-IX AS	5	80.0	100.0	100.0	80.0	80.0	80.0	100.0	60.0	60.0	60.0	80.0	60.0	78.3
Regional Means		61.5	88.0	62.4	51.3	25.8	33.7	50.0	43.3	28.9	31.7	74.1	61.5	

[1]Mb=labral brushes; P=pharynx; E=esophagus; GC=gastric caeca; 1-6=stomach segments; II=ileum; C=colon.
[2]Protions of gut obscured so that accurate observations could not be made.
[3]Abdominal segment.

E–6520). Before ligaturing or cautery, larvae were immobilized on a glass slide by withdrawing water from around them with filter paper strips. Larvae could then be gently oriented without damage using either a blunt probe or fine-tipped forceps.

Immediately following cautery, larvae lose a considerable amount of blood through the hole thus made in the cuticle. Many larvae were killed outright at the time of cauterization. It is not certain to what extent the gut itself may have been injured during these cautery experiments. Both ligatured and cauterized larvae were examined in distilled water either in the wells of micro slides or on ordinary glass slides at magnifications of 50x to 100x. Only the presence or absence of activity was observed and no attempts were made to detect possible quantitative alterations. Depression or inhibition of activity is arbitrarily defined here to mean that visible contractions in a given region were observed in less than 30% of the cases examined during the time specified.

The data in tables 2 and 3 show that the fore- and hindgut are much less affected by both ligaturing and cauterization than the midgut. The data support the earlier observation that activities in different regions of the alimentary canal are essentially independent; that is, that each region appears to be basically automatic and not greatly influenced by contiguous regions.

Ligaturing brings about a much greater depression of the rhythmical activities than cauterizing. It was, at first, assumed that this was not due to hypoxia since activity in some cases ceased posterior to the ligature where the tracheae looked normal and where the air supply and circulation of the blood were presumably or obviously normal; and since activity sometimes remained in regions where the tracheae had collapsed and the oxygen supply and circulation were presumably reduced or were obviously seriously impaired. However, when normal larvae were made hypoxic by sealing them under water, all of the activities of the intact gut were rapidly and markedly depressed or abolished. In some experimental *Anopheles* larvae where gut activity was absent, it was observed that strong body wall contractions did not induce contractions of the gut.

It is evident from table 3 that cauterization of the ventral ganglia tends to have a relatively small and localized abolishing effect at the maximum, so that the ganglia of the central nervous system of *Anopheles* larvae cannot be said to control or regulate most of the activities of the alimentary canal. Surely it seems significant that the gut of the *Anopheles* mosquito larva retains much activity after ligaturing the one clear-cut site of innervation at the anterior end of the gut. This finding suggests that the stomatogastric nervous system in this insect is not necessary for the rhythmical activities of the alimentary canal.

DISCUSSION

The outstanding feature of the alimentary canal of the *Anopheles* larva is the essential independence of its three major divisions: there is even automaticity within one given region. The truly remarkable thing is that all of these separate activities become coordinated in some as yet unknown (neurohormonal?) way so that food is brought into the organ, becomes distributed within it, and is finally ejected in a presumably orderly fashion. It is clear that while ingestion is directly dependent upon nerves (and it may be that egestion is, too), most of the rhythmical movements of the alimentary canal are fundamentally myogenic and we know nothing about how this activity is really regulated.

Table 3.—The effect of cauterizing various ganglia on the rhythmic activities of the alimentary canal of intact, 24-hour-old, male fourth-stage *Anopheles quadrimaculatus* larvae. Usually observed each larva 10 to 30 minutes; 27°–28° C.

Area cauterized[1]	Larvae used	Percentage with rhythmical activities in[2]												Mean activity of alimentary canal
		Mb	P	E	GC	1	2	3	4	5	6	II	C	
Ventral head	7	0	0	0	28.6	14.3	28.6	42.9	28.6	42.9	57.1	71.4	14.3	36.5
T-1	6	50.0	66.6	33.3	33.3	16.6	66.6	83.3	100.0	83.3	80.0	66.6	33.3	59.4
T-2	3	33.3	66.6	66.6	0	33.3	33.3	100.0	66.6	66.6	66.6	100.0	100.0	61.1
T-3	3	100.0	100.0	100.0	33.3	0	33.3	66.6	66.6	66.6	33.3	66.6	33.3	58.3
G-1	11	90.9	90.9	90.9	45.5	9.1	0	27.3	45.5	54.5	54.5	45.5	36.4	49.3
G-2	10	100.0	100.0	100.0	50.0	40.0	0	10.0	20.0	30.0	30.0	70.0	40.0	49.2
G-3	10	80.0	100.0	100.0	50.0	70.0	50.0	20.0	10.0	20.0	30.0	88.8	90.0	59.1
G-4	10	100.0	100.0	100.0	50.0	80.0	80.0	80.0	20.0	0	20.0	75.0	88.8	66.2
G-5	6	100.0	100.0	100.0	66.6	66.6	66.6	83.3	66.6	33.3	50.0	100.0	75.0	75.7
G-6	7	83.3	100.0	100.0	85.7	42.8	85.7	100.0	100.0	33.3	16.6	83.3	66.6	74.8
G-7	3	33.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	33.3	33.3	83.3
G-8	3	33.3	100.0	100.0	100.0	66.6	100.0	100.0	100.0	100.0	100.0	33.3	0	77.8
AS-9	2	50.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0	0	79.2
Regional Means		71.2	93.7	90.9	57.2	49.2	57.2	70.3	63.4	56.2	56.8	64.2	47.0	

[1]T-1 to T-3=Prothoracic, mesothoracic, metathoracic, metathoracic ganglia respectively; G-1 to G-8=ventral abdominal ganglia; AS=anal segment.

[2]Symbols same as in table 2.

Perhaps the myogenicity of the alimentary canal in *Anopheles* is unusual for the Diptera, since Gersch (1952) has demonstrated in *Corethra* (*Chaoborus*) normal regurgitation of food (from the midgut to the foregut) is reflexly released by taste stimuli (after removal of critical ganglia the regurgitations were stimulated). Duhr (1955) stated that the frequency of contractions of the cardia (valvular gland) were not dependent upon nerves but gives no good experimental proof. In a later paper Gersch (1955) convincingly showed that the nervous system partially regulates the gut movements of *Corethra*. Florey (1951) would have us believe that acetylcholine or some acetylcholine-like substance stimulates the corpora cardiaca to secrete adrenalin and that the adrenalin acts on the inhibitory nerves of *Corethra* to stimulate contractions and goes so far as to claim the *Corethra* gut is provided with cholinergic accelerator neurones. He showed with the crab that acetylcholine and adrenalin stimulated the hind-gut but that factor I arrests its activity. Gersch (1955) subsequently indicated that acetylcholine, eserine and histamine accelerate the *Corethra* gut while pilocarpine and adrenalin inhibit its movements. Ten Cate (1924) found that acetylcholine stimulated the *Dytiscus* fore-gut whereas adrenalin and atropine had little effect, but Kooistra (1949) did not notice much effect in *Periplaneta*, though he did note that mecholyl, eserine and HETP could have stimulating effects. It is evident that much physiological information will still be required before we shall have a satisfactory understanding of the regulating mechanisms of the movements within the alimentary canal of insects.

ACKNOWLEDGMENTS

I have much benefited from the advice of W. E. Bickley, M. F. Day, Vincent Dethier, Glenn Richards, R. E. Snodgrass, and Douglas Waterhouse, all of whom have critically read the manuscript.

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INFLUENCE OF PHOTOPERIOD ON OVIPOSITION BY THE ARMY CUTWORM, CHORIZAGROTIS AUXILIARIS (LEPIDOPTERA: NOCTUIDAE), IN AN INSECTARY[1]

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ABSTRACT

In the field in Alberta this insect emerges as a moth in June but does not lay its eggs until late August or September. A diapause, marked by prolongation of the pre-oviposition period, occurs in the adult stage. In an insectary, the mean durations of the pre-oviposition period at photoperiods of 18, 15, and 12 hours were 33.0, 46.5, and 27.3 days, respectively. The combined effect of restricted feeding and photoperiod in prolonging the pre-oviposition period is indicated.

The army cutworm, *Chorizagrotis auxiliaris* (Grote), terminates its feeding in southern Alberta in May and pupates soon after, and the moth emerges in June. Oviposition does not commence until late August or early September, 60 or more days after emergence (Seamans 1928). Records of captures of moths in light traps show that moths of the army cutworm are abundant after emergence, are relatively scarce during warm weather in July and August, and become more abundant again in late August. Dissection of moths showed that no ovarian development occurs in the first month after emergence and gravid females are not found until late August or later (Seamans 1928, Pepper 1931). The long interval between emergence and oviposition has been ascribed by Seamans (1928) and Walkden (1950) to a form of aestivation. Others have postulated that the moths migrate to nearby mountains to escape high temperatures during mid-summer (Pepper 1952, Chapman et al. 1955). In the laboratory it was found that the duration of the pre-oviposition period varied inversely with temperature and was affected by photoperiod and that food was necessary for the moths to subsist for extended periods of time (Jacobson and Blakeley 1959).

MATERIALS AND METHODS

An experiment was conducted in 1958 to determine the effect of various photoperiods on oviposition of the army cutworm at temperatures that prevailed in an insectary. Moths were obtained from laboratory-reared larvae so that emergence in the laboratory coincided nearly with emergence in the field. As emergence occurred over several weeks, the moths were stored at 10° C. with food until they were required. Commencing June 26 groups of five females and not fewer than four males were selected at random and placed in 1-pint glass fruit jars containing one-half inch of sifted soil. Four jars of moths were exposed to each of four photoperiods in a compartmented box, of the sort described by Jacobson and Blakeley (1959), and placed in a screened insectary. The moths were fed with a honey-water solution, which was replenished every second day. Commencing on the eighth day the soil in the jars was sifted daily for eggs and mortality of the moths was noted. The experiment was concluded on November 13

[1]Contribution from the Entomology Section, Canada Agriculture Research Station, Lethbridge, Alberta. Accepted for publication November 9, 1959.

(after 140 days) when mortality in all containers was between 98 and 100 percent.

RESULTS AND DISCUSSION

Table 1 shows that the duration of the pre-oviposition period was longest at a photoperiod of 15 hours, shortest at 12 hours, and intermediate at 18 hours and at the photoperiod that prevailed in the insectary. These data are similar to those obtained for diapause induction in other insects; the critical level at which diapause was induced in the red locust, *Nomadacris septemfasciata* (Serv.), was between 11 and 13½ hours (Norris 1959) and the transition point that induced diapause in European corn borer, *Ostrinia*[2] *nubilalis* (Hbn.), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was near 15 hours (Mutchmor and Beckel 1959, de Wilde et al. 1959).

TABLE 1.—Durations (days) of pre-oviposition periods of the army cutworm at various photoperiods

Photoperiod	Replicates				Mean
	1	2	3	4	
Prevailing light[a]	31	30	36	35	33.0
18 hours	35	32	30	35	33.0
15 hours	43	44	46	53	46.5
12 hours	26	26	26	31	27.3
L.S.D. at 5 percent level					3.7
At 1 percent level					5.3

[a]Approximately 17 hours during pre-oviposition period.

Several explanations can be advanced as to why the durations of the pre-oviposition periods in these experiments were all shorter than those that occur in the field. Mean temperatures inside the photoperiod cages in the insectary were 3° to 5° F. higher than outside the insectary. In these experiments food was continuously available whereas in the field feeding would be infrequent because of the necessity for the moths to forage and, sometimes, because of paucity of flowers. De Wilde et al. (1959) found, with the Colorado potato beetle, that a shortage of food was an added factor in bringing about diapause.

The photoperiods in the test described above differ obviously from those in other situations. Moths hiding in buildings and in crevices in the ground would probably be influenced by photoperiods somewhat shorter than those that prevail in the open.

Mortality of females was associated with durations of the pre-oviposition periods. After 60 days, mortality at the 12- and 15-hour photoperiods was 84 and 54 percent, respectively.

The extension of the pre-oviposition period observed in these rearings suggests that the similar pre-oviposition period observed in the field may be influenced by length of day. A diapause evidenced by a prolonged pre-oviposition period occurs in the adult stage. The combined effect of restricted feeding and photoperiod in prolonging the pre-oviposition period requires further investigation.

[2]Use of the generic name *Ostrinia*, rather than the more familiar *Pyrausta*, follows Marion (1957).

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THE RELATIVE EFFECTS OF COCOON SUBMERGENCE ON THE MORTALITY OF THE LARCH SAWFLY, PRISTIPHORA ERICHSONII (HYMENOPTERA: TENTHREDINIDAE), AND ITS PARASITE BESSA HARVEYI (DIPTERA: TACHINIDAE)[1]

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ABSTRACT

Laboratory experiments on effects of submergence of larch sawfly cocoons, immediately after the overwintering period, confirmed earlier reports on the resistance of the eonymphal stage to flooding. An ichneumonid parasite, *Mesoleius tenthredinis* Morley, appeared able to survive flooding as well as the host, but the tachinid *Bessa harveyi* (Tnsd.) was found susceptible even when the flooding was of short duration. The early inception of growth and the rapid development of *B. harveyi* maggots, and the resultant active metabolism, seemed to account for its susceptibility to submersion. Sawfly larvae parasitized by this fly were found to have a higher rate of oxygen consumption than unparasitized larvae. Susceptibility to submersion seems to be a factor limiting the effectiveness of *B. harveyi*, and may account for its reported sporadic distribution in localized areas.

Upon completion of feeding, larvae of the larch sawfly, *Pristiphora erichsonii* (Htg.), drop to the ground from the host tree, burrow into the moss or duff, and spin their cocoons. A period of approximately 10 months is spent in the cocoon, from late summer until the following spring. Most of the larvae overwinter as eonymphs in diapause, and, in the following spring, continue development through pronymphal and pupal stages prior to adult emergence. During this extended period in the ground the cocoons are subject to submersion caused by fluctuating water levels in their bog habitat. Flooding frequently occurs during the spring run-off.

Lejeune et al. (1955) studied the effects of such inundation on the various developmental stages that occur within the cocoon. They demonstrated that whereas pre- and postdiapause stages could survive only short periods of submergence, the diapausing eonymphs could tolerate relatively prolonged periods under such conditions. The degree of susceptibility of the various developmental stages was correlated with their relative rates of oxygen consumption.

One of the principal parasites of the larch sawfly in Manitoba and Saskatchewan is the tachinid *Bessa harveyi* (Tnsd.). In most instances this parasite overwinters within the host as a first-instar maggot and completes its development the following spring and summer. There are three larval instars. The fully developed third-instar larva emerges from the host larva and usually forms its puparium outside the sawfly cocoon.

During its larval stages within the host the parasite is subject to the same flooding conditions as is the sawfly. The experiments described in this paper were undertaken to determine the relative tolerance of the parasite and host to the effects of submersion. The study was concerned with the effects of continuous inundation of cocoons immediately following the overwintering period.

METHODS AND MATERIALS

1. *Experimental treatment of cocoons.*—The cocoons used in these experiments were collected in October 1957 in a tamarack bog north of Riverton, Manitoba. Following collection they were stored in moist sphagnum moss at an average temperature of 2.5° C. until May 5, 1958, when the experimental treatments commenced.

Duplicate experiments were conducted at 10° C. and 15° C. Although the environmental temperatures vary considerably, the former temperature is close to the average temperature to which cocoons in the field are exposed during the spring development period. The cocoons subjected to submergence treatments were placed in jelly jars between pads of glass wool, in groups of 50 cocoons per jar. The jars were ballasted and submerged in 4½-gallon pyrex containers filled with distilled water. Four-hundred and fifty cocoons were submerged in each pyrex container. At each temperature, groups of approximately 300 cocoons were subjected to submergence treatments of 10, 20, and 30 days duration, respectively. Following treatment, cocoons were incubated at the respective temperatures and emergence of sawflies and parasites was recorded daily. In addition to *B. harveyi*, a few adults of the ichneumonid *Mesoleius tenthredinis* Morley emerged from the cocoons and these were also recorded. An untreated control group of approximately 300 cocoons was incubated at each temperature.

[1]Contribution No. 587, Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada. Accepted for publication November 28, 1959.

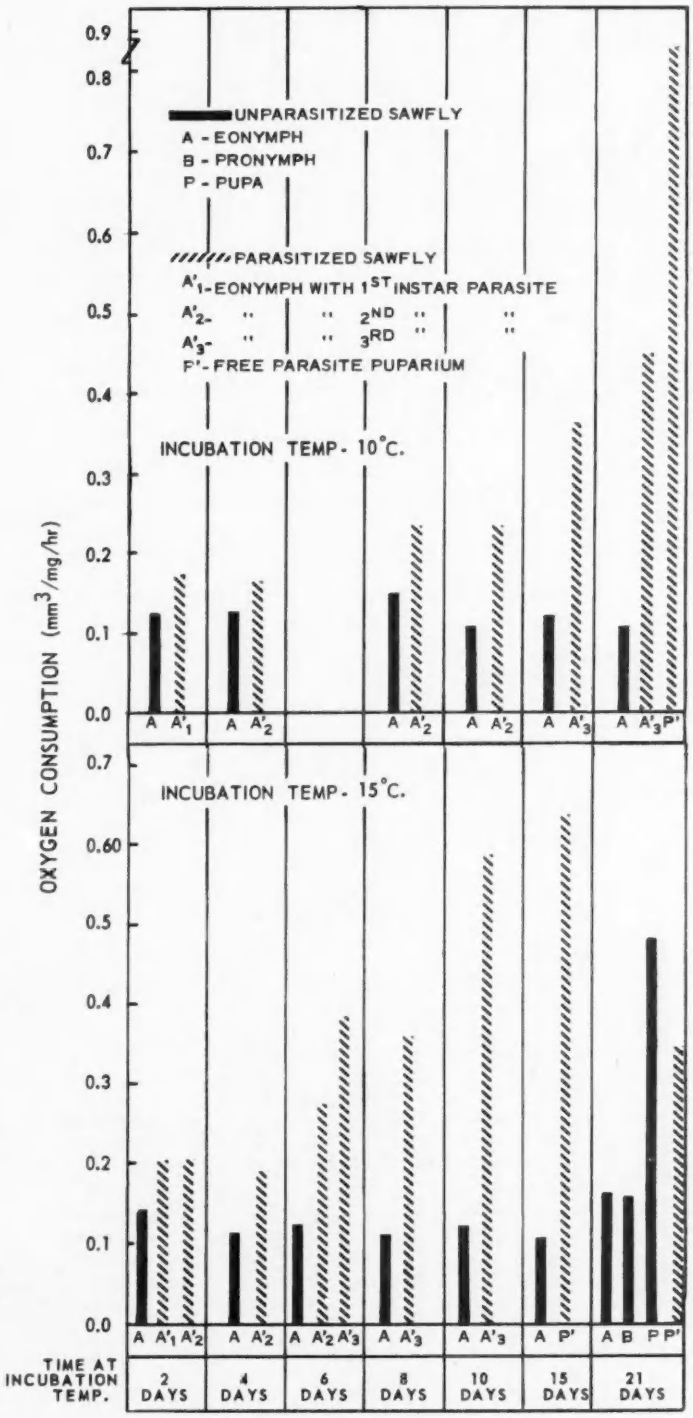

FIG. 1.—Average rates of oxygen consumption (per mg. wet wt.) of unparasitized larvae and pupae of the larch sawfly and of larvae parasitized by *Bessa harveyi* (Tnsd.) determined after various periods of incubation at 10° C. and 15° C.

TABLE 1.—The effects of cocoon submergence treatments on the survival of the larch sawfly and its parasites *Bessa harveyi* and *Mesoleius tenthredinis* Morley.

Incubation temperature (° C.)	Treatment	Total number of cocoons	Emergence of larch sawfly and parasite adults (% of total cocoons)			Living unemerged sawfly larvae at conclusion of experiment (% of total cocoons)		Corrected percent total mortality[a]
			P. erichsonii	*B. harveyi*	*M. tenthredinis*	Living eonymphs	Living pronymphs	
10	Untreated	276	69.9	9.8	0.4	0.0	0.4	——
10	Submerged 10 days	300	71.0	2.0	1.0	1.0	0.7	5.9
10	Submerged 20 days	299[b]	70.9	0.0	1.0	0.3	0.3	9.4
10	Submerged 30 days	299	68.2	0.0	0.3	1.0	1.3	12.0
15	Untreated	266	60.1	12.4	2.3	0.0	0.0	——
15	Submerged 10 days	286	61.2	0.3	1.4	0.0	0.0	15.9
15	Submerged 20 days	293	58.0	0.0	0.7	1.7	0.0	19.3
15	Submerged 30 days	301	38.9	0.0	1.0	2.6	0.0	43.2

[a]Calculated from Abbot's formula.
[b]Includes one cocoon from which an unidentified hymenopterous parasite emerged.

On September 10, after a considerable period during which no further adult emergence occurred, the cocoons were examined. The numbers of living sawfly larvae in prolonged diapause and numbers of dead larvae were recorded. The condition of the dead larvae made it impossible to determine whether or not they had been parasitized by *B. harveyi*. Most of the mortality in the control group was due to disease caused by fungi and other pathogenic organisms.

Information concerning the stage of development of *B. harveyi* at the time of death due to submergence was obtained by dissecting parasitized larvae from cocoons submerged for varying lengths of time. For this purpose a group of 150 cocoons was removed from cold storage on May 14, 1958. A sample of these cocoons was dissected immediately to determine the stages of development of the host and parasite. The remainder were subjected to submergence treatments at 15° C. Samples of the submerged cocoons were taken after 10, 15, and 20 days of treatment. They were held for 4 days to allow for recovery from the comatose state which is induced by submergence. The larvae were then dissected to determine the instars and condition of the parasite larvae.

2. *Respiration measurements.*—The rates of oxygen consumption of normal and parasitized sawfly larvae were determined at various intervals during the first 21 days of incubation at 10° C. and 15° C. This covered the period during which the immature stages of *B. harveyi* were encountered. All measurements were made at room temperature (22.5° ± 1.5° C.), using capillary respirometers similar in design to that described by Smith and Douglas (1949).

The larvae were held at room temperature for approximately 3 hours before respiration determinations were made. In most cases measurements were made over a period of 3 hours. Readings were made at 15-minute intervals. Parasitized host larvae were dissected following the experiments and the larval instars of *B. harveyi* determined on the basis of the morphological features described by Hawboldt (1947).

RESULTS AND DISCUSSION

1. *Effects of submergence treatments on the mortality of host and parasites.*—Data pertaining to the effects of the experimental treatments on the survival of the larch sawfly, the tachinid *B. harveyi*, and the ichneumonid *M. tenthredinis* are summarized in table 1. At 10° C. submergence of cocoons for periods up to 30 days caused very little sawfly mortality. There was a marked decline in emergence of *Bessa harveyi* adults after 10 days submergence, and mortality of this parasite was complete in the group submerged for 20 days. At 15° C., after submergence for periods up to 20 days there was little sawfly mortality but considerable mortality resulted from the 30-day treatment. A single *B. harveyi* survived the 10-day submergence treatment and mortality was complete after 20 days of inundation.

At the commencement of the treatments practically all the larvae were in the eonymphal

stage. The experiments demonstrated the high degree of tolerance of the sawfly to submergence at this stage of development. This was in agreement with the results of Lejeune et al. (1955), whose field experiments indicated that approximately 50 days of continuous flooding in the spring were required to cause complete mortality of overwintered eonymphs. The increased mortality at the higher temperature reflects the effect of temperature on metabolism and development. Since diffusion of oxygen from the water into the cocoon may occur, it is possible that the higher aqueous-oxygen tension at the lower temperature may also be a factor influencing survival.

In striking contrast to the low mortality of the sawfly was the susceptibility of *B. harveyi* to the effects of submergence. Development of this parasite resumes very soon after the cocoons are removed from the overwintering temperature (2.5° C.) to 10° C. or 15° C. At 10° C. the mean number of days to adult emergence of *B. harveyi* was 64.4 days (range 56 to 74) and at 15° C. it was 35.7 days (range 30 to 41). As approximately 50 percent of this time is spent within the puparium, it is evident that larval growth is rapid. The average weight of third-instar maggots is approximately 30 times that of first-instar larvae. The metabolic demands during the period of larval growth must be considerable. In view of the known correlation between the relative susceptibility to submergence of developmental stages of the sawfly and their rates of oxygen consumption, it was logical to investigate the relative rates of respiration of parasitized and unparasitized sawfly larvae. The results of the respiration experiments and their bearing on parasite mortality are discussed in a following section of this paper.

Data from dissections undertaken to determine the stage of development at which mortality of *B. harveyi* occurred following submergence are summarized in table 2. Several cases of multiple parasitism are not included in the tabulation because of the complicating factors involved where more than one parasite is present. Although the number of larvae that were dissected was small, the results are conclusive. All the larvae in the submergence treatments were dead. Their necrotic state was evidenced by their brownish discoloration. In no case did development proceed beyond the second larval instar before death. Some mortality occurred during the first stadium, but most of the parasites succumbed during the second stadium.

Only a very small number of *M. tenthredinis* adults emerged from the cocoons used in these experiments. It was difficult, therefore, properly to evaluate the effects of the experimental treatments on this parasite. It is apparent, however, that *M. tenthredinis* was much less affected by inundation than was *B. harveyi*. At both 10° C. and 15° C. *M. tenthredinis* adults emerged from cocoons that had been submerged for 30 days.

There is some disagreement in the literature concerning the larval instar in which *M. tenthredinis* overwinters in its host. In Manitoba and Saskatchewan, however, Muldrew (1953a) found that most of the parasites overwintered in the third and fourth stadia. It is likely that larvae overwintering in these advanced stages do not commence development as soon following removal from the cold as is the case with *B. harveyi*. This may account for the difference in the susceptibility to submergence of these two parasites.

TABLE 2.—Record of dissections of sawfly larvae from cocoons submerged for various lengths of time at 15° C., indicating instars and condition of *Bessa harveyi* larvae

No. of days cocoons submerged	Developmental stage of host	No. of *Bessa harveyi* larvae			
		First instar		Second instar	
		Living	Dead	Living	Dead
0	Eonymph	4	—	1	—
10	Eonymph	—	—	—	6[a]
10	Pronymph	—	1	—	2
15	Eonymph	—	3	—	3
15	Pronymph	—	—	—	5[b]
20	Eonymph	—	2	—	2[b]
20	Pronymph	—	1	—	2

[a]One parasite larva encapsulated by host.
[b]Two parasite larvae encapsulated by host.

2. *Respiration measurements.*—The data from the respiration measurements are summarized graphically in figure 1. Each bar represents the mean oxygen consumption derived from measurements of from two to six individuals. In spite of individual variation, in no case was there an overlapping of the ranges of the parasitized and unparasitized larvae for a given stage of development of host and parasite.

In the cocoons incubated at 10° C. all larvae were in the eonymphal stage during the 21-day period in which respiration measurements were made. Throughout this period the oxygen consumption of the unparasitized eonymphs remained at a near-uniform low level. The average rate of respiration is in close agreement with that reported by McDonald and Brown (1952) for overwintering eonymphs in diapause and after 20 days incubation at room temperature. At 15° C. all the sawfly larvae were in the eonymphal stage during the first 15 days of incubation. The rate of oxygen consumption during this period was similar to that noted for the larvae incubated at 10° C. At 21 days some pronymphs and

pupae were encountered. The respiration rate of the pupal stage was approximately four times that of the eonymph.

When the first respiration measurements were made, on the second day at the incubation temperature, the oxygen consumption of the parasitized sawflies was appreciably greater than that of unparasitized host larvae. At this time most of the parasite larvae were in the first stadium. First-instar *B. harveyi* larvae are very minute, and averaged 1.3 mm. in length and 0.3 mg. in weight. The average weight of host larvae was 61 mg. It is improbable that the appreciable difference in the respiration of the parasitized and unparasitized sawfly larvae could be entirely attributable to the metabolic activity of the first-instar parasite maggot. It is more logical to assume that at least part of the difference is due to a stimulated metabolism of the host induced by the presence of the developing parasite.

As the parasite continues its development there is a progressive increase in the respiratory rate of the host-parasite unit. As noted above, growth of the parasite progresses very rapidly, particularly at 15° C. Third-instar larvae were first encountered on the sixth day at this temperature. This rapid growth is reflected in a sharp rise in the rate of oxygen consumption of the host-parasite unit. A somewhat slower rate of increase in respiration is noted at 10° C.

By the time the third-instar *B. harveyi* larva has completed its growth it has consumed a considerable portion of the host viscera. At this stage a major part of the total respiration must be due to the parasite.

The difference in the relative degrees of susceptibility of *B. harveyi* and the larch sawfly is clearly related to the difference in their rates of metabolism during the early part of the incubation period. In contrast to the rapid initiation of development by *B. harveyi* the post-diapause development of the sawfly proceeds very slowly. In addition to this normal delay in inception of development the sawfly showed a considerable degree of adaptability in its capacity to arrest development to some degree when adverse conditions are imposed by submergence. Adult emergence in the control group at 10° C. extended over 64 days. In the 20-day submergence group this period was reduced to 41 days. The submerged larvae were apparently able to proceed to a certain stage before their further development was arrested. Once favourable conditions again prevailed, development resumed. Thus there was a marked peak in the adult emergence curve for the submerged cocoons which did not occur in the controls. *B. harveyi* larvae were apparently unable to arrest their development, and succumbed when their oxygen demands could not be fulfilled.

CONCLUSIONS

Although *B. harveyi* is considered to have contributed to the decline of larch sawfly populations in several areas in Canada (Lejeune and Hildahl 1954; Reeks 1954), a number of factors have been reported to limit its effectiveness. These are: sloughing of eggs and maggots, premature dying, encapsulation of maggots, superparasitism and poor synchronization with the host (Reeks 1954; Muldrew 1953b). To these should now be added its relatively high susceptibility to spring flooding in contrast to the adaptability of the larch sawfly in this respect.

It has been noted that parasitism by *B. harveyi* is frequently of sporadic occurrence (Reeks 1954; Lejeune & Hildahl 1954). The percentage parasitism may be very high in one tamarack stand and very low in a neighboring one. No explanation has apparently been found for such discontinuities in distribution. As the water levels in adjacent tamarack stands may differ considerably it is possible that this may be the factor that accounts for the observed localized variations in incidence of parasitism. Some recent incidental evidence obtained from field studies supports the conclusions drawn from these experiments concerning the importance of flooding as a mortality factor for *B. harveyi*. In investigating the distribution of larch sawfly cocoons with respect to predation by small mammals, Buckner (1959) noted that in two "wet" tamarack bogs the percentage of old cocoons with *B. harveyi* emergence holes was significantly lower in the deepest layer of moss.

Although the importance of water levels and flooding in the ecology of the larch sawfly is well established (Graham and Satterlund 1959; Lejeune et al. 1955), evidence is presented here for the first time of a differential effect on the sawfly and its parasite *B. harveyi*. These experimental results indicated the need for further study of this problem in the field, and such a study is now being carried on by personnel of this laboratory.

ACKNOWLEDGMENTS

I wish to thank W. A. Reeks and J. A. Muldrew for their critical review of the manuscript. It is a pleasure to acknowledge the assistance of D. M. Chapman in various phases of this study.

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THE GENUS SINELLA BROOK (COLLEMBOLA: ENTOMOBRYIDAE) IN NEARCTIC CAVES[1]

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ABSTRACT

The nine species known to occur in Nearctic caves are keyed and seven of these are described and illustrated. The other two are edaphic forms, rarely found in such situations. Except for *S. cavernarum* (Packard) (=*Entomobrya lucifuga* Folsom), which ranges from Pennsylvania to eastern Missouri, each truly cave-dwelling species has a very limited known distribution within an overall area extending from western Virginia to the Mississippi River and from south-central Indiana to northern Alabama. New species are: *S.* **alata,** *S.* **avita,** *S.* **barri,** and *S.* **krekeleri.** *S. cavernarum* var. *basidens* Bonet is raised to specific status, and *Parasinella* Bonet is synonymized under *Sinella* Brook.

Packard (1888) described the first Nearctic species of this group from Wyandotte Cavern, Indiana, as *Degeeria cavernarum*. Bonet (1931) catalogued it in *Entomobrya* Rondani, subgenus *Parasinella*, and added a "variety" which he called *basidens*. Both *Parasinella* and *basidens* were nomina nuda in that work, but were validated 3 years later (Bonet 1934). Still later, Bonet (1942) elevated *Parasinella* to generic status, but Gisin (1944) relocated it as a subgenus of *Sinella* Brook, 1882, a disposition which has generally been accepted since that time. However, as will appear below, *Parasinella* can not be maintained even as a subgenus of *Sinella*.

Wray (1952) described a second Nearctic cave species, *Sinella hoffmani*. The present paper presents a more extensive investigation of the Nearctic cave fauna of the genus, which has resulted in the discovery of a species complex consisting of *S. cavernarum*, *S. hoffmani*, *S. basidens* (here raised to specific status), and four new species. This is a greater variety of forms than is known from all the rest of the Western Hemisphere, and closely parallels the development of the genus in Japanese caves. The material examined was largely collected by Thomas Barr, Donald Burton, Oscar Hawksley, Leslie Hulbricht, and Carl Krekeler. I wish to extend my thanks to all of them, and also to acknowledge the aid of Jerry Tecklin, who did most of the preliminary work for this study.

The different species in this complex show a striking combination of great organ modifications and basic similarity. Thus, while all have a bidentate mucro of similar shape, the unguis varies so greatly that almost all the species can be separated on this basis alone. There is a good deal of geographical isolation among the species. However, some sympatry does occur, and in these localities there is no evidence of interbreeding. On the contrary, even where two species have some similarities, these usually are least marked in sympatric populations. As a result, there can be no question that a single, polytypic species is here involved.

The known distributions of the various species are shown on maps 1 and 2. The doubtful localities indicated were due to an accident in handling specimens but are very probable, as all records in serious doubt were discarded. No readily available system will explain the distribution of these species. They do not seem to be limited to specific small watersheds, nor by

[1]The work reported in this paper was made possible by N.S.F. grant G-4563. Accepted for publication November 25, 1959.

specific cave systems, and at least a few species appear to violate any kind of barrier that can be postulated.

In general, the group (or most of it) appears limited to the area south of the Great Lakes and east of the Mississippi River. Extensive collections from Iowa, Wisconsin, and Minnesota have failed to turn up a single specimen. *Sinella cavernarum* has been taken in eastern Missouri and also in Pennsylvania; it has been widely reported elsewhere, but so far none of these reports have been validated.

Within these rough boundaries the species appear to be limited by point of origin and by competition. The rarity of collections of two species from the same cave, even if the species are extensively sympatric, may be explicable upon the ground of intense competition. The fact that troglophile species such as *S. caeca* Schött and *S. curviseta* Brook never occur in caves already occupied by a species of this group further indicates that competition among the members of the genus is intense.

The extremely local distributions of most of the species indicate that their movement is very limited. This is quite in contrast to the continent-wide distributions characteristic of most epigeic Collembola. That the barriers to travel in this group are not absolute is evident from the wide range of *S. cavernarum*, a species which is unique also in its having secondarily invaded a micro-cavernicole habitat—the grave.

A general consideration of the evolution of the species of the group is beyond the scope of the present paper. However, the most primitive and the most highly specialized of its species show the most limited distribution. This supports the thesis of intense competition among the members of the group, while offering additional evidence that evolution is going on here at a rapid rate.

SYSTEMATIC PORTION: GENERAL

Five species of the genus *Sinella* Brook, 1882, have previously been recorded from North American caves. Two of these, *S. caeca* Schött, 1896, and *S. curviseta* Brook, 1882 (the type species of the genus), are epigeic forms with either trogloxene or troglophile habits. *S. caeca* appears to be established in a number of western caves, but rarely has been recorded from caves in the eastern states. The few records of *S. curviseta* from caves probably represent temporary infestations. At any rate, neither of these species is a regular inhabitant of the area occupied by the primarily cavernicolous species, which are the ones considered in detail in this paper.

These cave-dwelling species have a large number of characteristics in common. The same basic type of chaetotaxy occurs throughout the group, with small population variations or individual aberrations, none of which are of specific significance. The mucro has a bidentate structure, with a clear but relatively small basal spine; the apical tooth is very elongate and curves upward strikingly (see fig. 8). The body is typically entomobryiform (fig. 1), except in a few species where it becomes attenuated. The antenna lacks an apical bulb (see fig. 54). One of the most striking characteristics is the development of peculiar, flattened setae on the apex of the third antennal segment. These typically are shaped like ping-pong paddles, often with the supporting rod along one margin (see fig. 23). They clearly are related to the sensory pegs seen elsewhere in the genus, and apparently are a cave modification as they occur also in the unrelated cave-dwelling *Sinella* of Japan. Other setae are of the same five types normally found in this genus and in *Entomobrya*. The large, clavate, ciliate setae are dense upon the first thoracic segment and the dorsum of the head, and are found also on the basal segment of the antenna. Eyes are always absent, and pigment is limited to scattered, red patches.

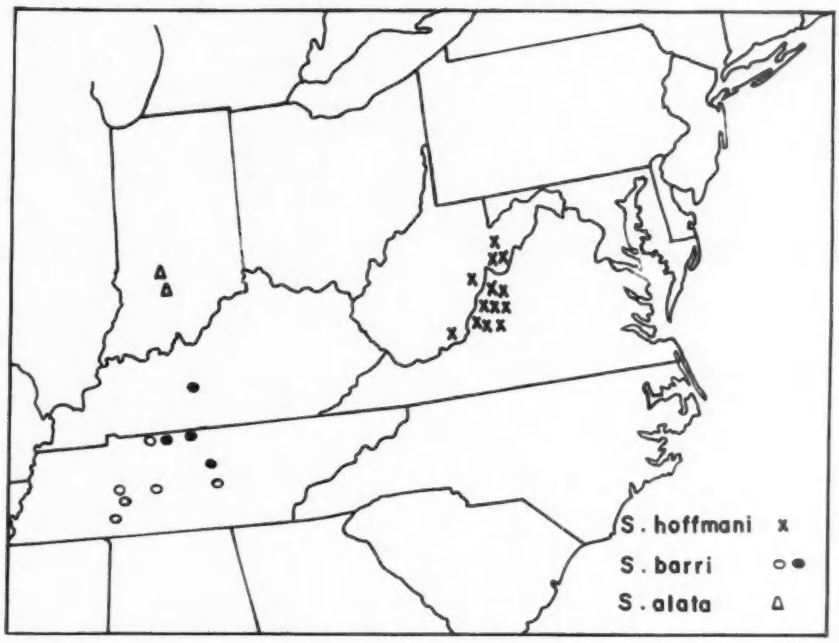
Many of the characteristics listed above, it may be argued, represent adaptations to cave life. However, in the cases of the mucro and the chaetotaxy this obviously is not true, as both of these are strikingly different from those of the Japanese cavernicole *Sinella*. I feel that these similarities, combined with the generally related male genital plates, argue for a single, common origin for the group.

Bonet (1942) elevated *Parasinella* to generic rank for "species related to *Entomobrya*, without scales, eyes, or pigment, with wing-like teeth on the claws, and without a double row of smooth setae on the tibiotarsus." The divisions thus indicated violate every bit of interrelationship we have, and the species dealt with here can not be fitted into these generic limits. I therefore consider *Parasinella* as a synonym of *Sinella*.

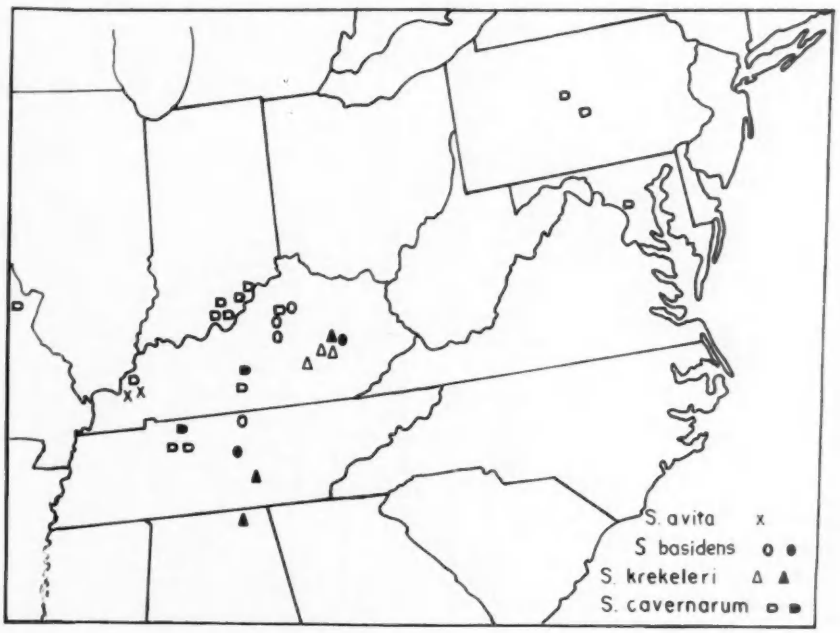
The types of the new species described here are in the author's collection.

KEY TO CAVERNICOLE NEARCTIC SINELLA

1. Mucro falcate . . . . . **caeca**
   Mucro bidentate 2
2. Basal spine extending nearly to mucronal apex . . . . . **curviseta**
   Basal spine extending to level of anteapical tooth . . 3
3. Inner ungual teeth three or four 4
   Inner ungual teeth two 6
4. Internal surface of tibiotarsus with double row of "smooth" (finely striate) setae . . . . . **barri**
   Tibiotarsal setae all plainly ciliate, except for subapical smooth one 5
5. Inner teeth four; all tenent hairs strongly clavate . . . . . **avita**
   Inner teeth three; tenent hairs usually pointed . . . . . **cavernarum**
6. Antennae more than 3 times as long as head . . . . . **krekeleri**
   Antennae less than 2½ times as long as head 7
7. Internal surface of tibiotarsus with double row of "smooth" (finely striate) setae . . . . . **hoffmani**



MAP 1.—Distribution of *Sinella hoffmani*, *S. barri*, and *S. alata*. The solidly filled symbols represent somewhat questionable records (see text).



MAP 2.—Distribution of *Sinella avita*, *S. basidens*, *S. krekeleri*, and *S. cavernarum*. The solidly filled symbols represent somewhat questionable records (see text).

Tibiotarsal setae all plainly ciliate, except for single, subapical smooth one 8

8. Distance from inner base of unguis to apex of largest basal tooth more than one-half the length of empodial appendage ..... **alata**
Distance from inner base of unguis to apex of largest basal tooth less than one-half the length of empodial appendage ..... **basidens**

Sinella avita, new species

Figures 1–8

Facies typical for the genus. Head broadly oval. Antennae slightly less than twice as long as head, first segment cylindrical, the others slightly ellipsoidal; apex of third segment with a "sense" organ of two large, oval pegs, opposite side of apex with seven short pegs which are smooth, narrowly fusiform, and about one-third as long as the neighboring ciliate setae; fourth segment with a subapical, paddle-shaped peg which is thickened along one margin. External differentiated seta of labial appendage definitely surpassing apex of same papilla, slightly thicker than apical seta. Internal setae on tibiotarsus only slightly differentiated, all (except a single, smooth, subapical one) with well-developed ciliations. Unguis with four well-developed internal teeth, all relatively small; most basal tooth slightly distal to mid-level of unguis, second basal tooth definitely smaller and more apical in position; small external tooth present, located at about one-third the distance from base to apex of claw. Empodial appendage acuminate, its apex more sharply angled. Tenent hair broadly clavate. Apical tooth of mucro only slightly longer than subapical one. Male genital plate with all setae flattened, heavy, and angularly truncate, the basal seta larger and more sharply truncate than the others.

Measurements[2] *of holotype:* Head, maximum 0.52; antennal segments 1–4, respectively, 0.13 (0.11), 0.26, 0.22, 0.31 (0.30); second thoracic segment 0.22; third thoracic segment 0.17; abdominal segments 1–6, respectively, 0.09, 0.17, 0.16, 0.59, 0.15, 0.05; manubrium 0.40; dens 0.621 hind femur 0.33; hind tibiotarsus 0.51; hind unguis 0.54 (0.51); hind empodial appendage 0.027; hind tenent hair 0.042; mucro 0.018.

Type locality: Kinnin Cave, Crittenden Co., Kentucky (L. Hulbricht, collector). Also taken from Potato Cave, Livingston Co., Kentucky.

The paddle-shaped peg on the fourth antennal segment was not visible on all specimens, but careful search showed it present, and varying but little, upon 80 percent of those examined thus far. The more finely ciliated setae on the tibiotarsus varied somewhat, but they always were plainly ciliated.

This species is quite like *S. cavernarum* in the structure of the male genital plate, but is readily distinguished by the structure of the unguis, the labial seta, and the "sense" organ of the third antennal segment. *S. avita* seems the most primitive members of the group, and probably is near the ancestral form.

Sinella cavernarum (Packard)

Figures 9–16

Degeeria cavernarum Packard 1888: 66.
Entomobrya lucifuga Folsom 1902: 365.
E[ntomobrya]. (Parasinella) cavernarum, Bonet 1931: 276 and 383; Bonet 1934: 365–8.
Parasinella cavernarum, Bonet 1942: 56.
Sinella (Parasinella) cavernarum, Gisin 1944: 69.

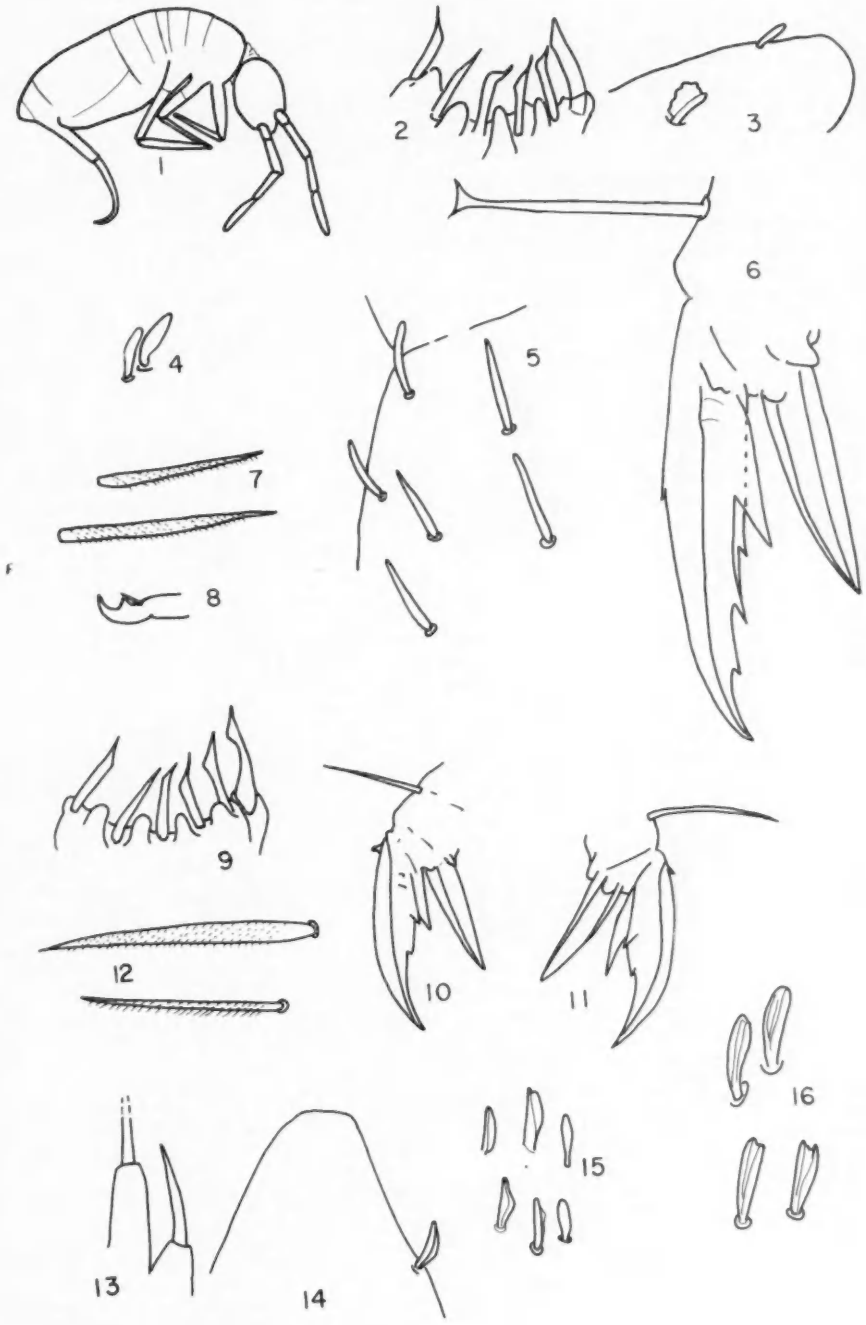
Facies typical for the genus. Head oval. Antennae about twice as long as head, first three segments subcylindrical, fourth segment truncate-fusiform; apex of third segment with "sense" organ of two flattened paddles, their supporting thickening usually single, along one margin; opposite side of apex without notably differentiated curved setae; fourth segment with a differentiated sensory peg below apex. Internal differentiated seta of labial appendage almost attaining level of apex of same papilla. Unguis with one of the inner basal pair of internal teeth considerably larger than the other; a small, median tooth at end of a short lamella which extends slightly beyond second basal tooth; a small, but definite, tooth externally at base of unguis. All internal setae on tibiotarsus (except a single, subapical smooth one) definitely ciliate, but with a clear distinction between more finely and more coarsely ciliate types. Empodial

[2]In this and other descriptions all measurements are in millimeters. A discrepant measurement for the second of a pair of organs is enclosed in parentheses.

EXPLANATION OF PLATE I

FIGURES 1–8: *Sinella avita* (all ×800 unless otherwise stated). FIG. 1.—Habitus (holotype, ×18. FIG. 2.—Male genital plate (holotype). FIG. 3.—Apex of fourth antennal segment (paratype). FIG. 4.—"Sense" organ on apex of third antennal segment (paratype). FIG. 5.—Thick, blunt setae on opposite side of apex of third antennal segment (same paratype). FIG. 6.—Hind unguis (paratype). FIG. 7.—Finely and coarsely ciliated setae, internal surface of tibiotarsus (paratype). FIG. 8.—Mucro (holotype), ×350.

FIGURES 9–16: *Sinella cavernarum* (all Indiana specimens, and all ×800 unless otherwise stated). FIG. 9.—Male genital plate (specimen from Steerstreeter Cave). FIG. 10.—Hind unguis (specimen from Shelby Cave), ×350. FIG. 11.—Hind unguis (specimen from Fourteen Mile Cave), ×350. FIG. 12.—Finely and coarsely ciliated setae, internal surface of tibiotarsus (specimen from Shelby Cave). FIG. 13.—External labial seta and same papilla (specimen from Shelby Cave). FIG. 14.—Apex of fourth antennal segment, showing subapical peg (specimen from Indian Cave). FIG. 15.—Variations occurring in the shape of the subapical peg. FIG. 16.—Apical "sense" organs of third antennal segment (above, specimen from Fourteen Mile Cave; below, from Steerstreeter Cave). All magnifications are approximate.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appendage acuminate, with a somewhat ragged external margin. Tenent hair pointed or weakly clavate. Setae of male genital plate small, arising between well-developed papillae; basal seta swollen basally and truncate apically, clearly differentiated from the others, which are angularly truncate.

Type locality: Wyandotte, Cave, Harrison Co., Indiana. Known distribution includes Pennsylvania, District of Columbia (grave), southern Indiana, Kentucky, and north-central Tennessee; rare (two collections) in east-central Missouri. This is the most widespread member of the genus.

S. cavernarum offers several characters upon which it can be distinguished from its relatives, but the peculiar structure of the male genital plate is probably the best criterion. This appears to be the most variable species of all that were examined in the present study. The subapical peg of the fourth antennal segment is extremely variable (see fig. 15). It can be seen on only about one-half of the specimens; but in view of the extremely complicated setae present on this segment, failure to find this organ does not necessarily indicate its absence. The median internal tooth of the unguis may be absent, but in such cases the blunt end of a median lamella can be seen as evidence of the three-toothed condition. There is a great deal of variation in the difference between the more finely and the more coarsely ciliate internal setae on the tibiotarsus. In some cases this is striking, in others barely perceptible, but high magnification always shows that all internal setae (except the single subapical one) are clearly ciliate. The apical "sense" organs of the third antennal segment sometimes have the supporting thickening bifurcate, the organ thus becoming triangular.

The synonymy of *S. lucifuga* (Folsom) with *S. cavernarum*, first suggested by Bonet (1934) then later stated by him (1942) on the basis of Folsom's description, has been questioned by some subsequent workers. My examination of the type series of *lucifuga* has revealed no significant differences between those specimens (collected from the grave) and cave-dwelling *cavernarum*. A more extensive investigation of the fauna of the grave is required before we can assess the extent to which *S. cavernarum* has invaded this microcavernicole habitat.

Sinella alata, new species

Figures 17–25

Facies typical for the genus; color dull yellow to off-white. Head almost round. Antennae about 1½ times as long as head; first segment subcylindrical, second and third segments truncately conical, fourth segment ellipsoidal; apex of third segment with a "sense" organ of two paddle-shaped pegs, each with a central thickening and entire margins; fourth segment with a subapical, bifurcate peg having a short, curved rod situated directly beside it. Labial appendage with external differentiated seta just reaching level of apex of same papilla, about 1½ times as thick as apical seta. Internal tibiotarsal setae with little or no clear distinction between more coarsely and more finely ciliated types. Unguis with small external tooth near base; internal teeth limited to basal pair, of which one is very large, the other of normal size. Empodial appendage acuminate, but with a distinct tooth on external margin. Male genital plate with basal seta larger than the others, blunted apically, the others acuminate and slender. Mucro normal for the group.

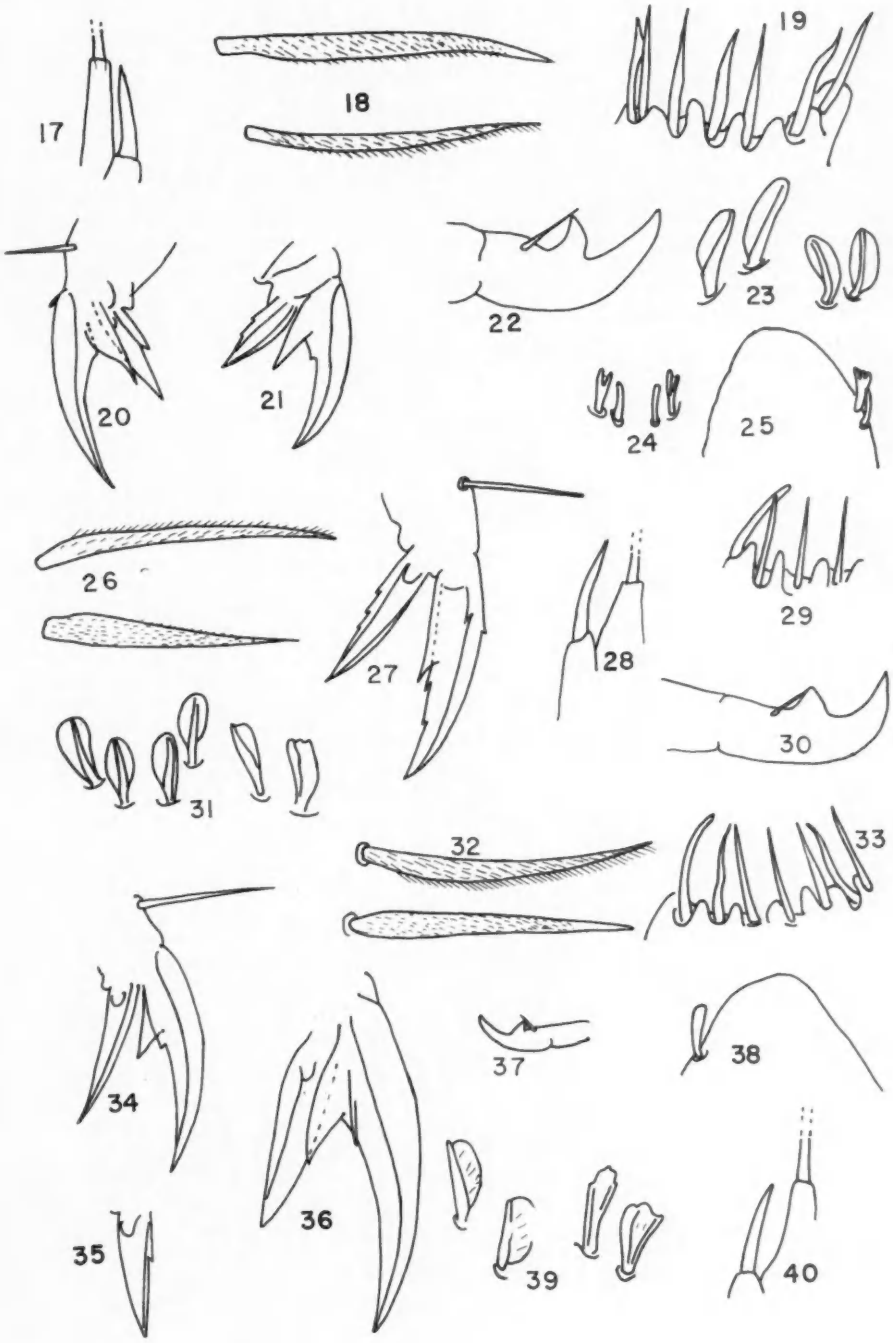
Measurements of holotype: Head, maximum, 0.52; antennal segments 1–4, respectively, 0.15 (0.13), 0.24 (0.23), 0.28 (0.26), 0.35; second thoracic segment 0.24; third thoracic segment 0.18; abdominal segments 1–6, respectively, 0.11, 0.15, 0.20, 0.78, 0.14, 0.11; manubrium 0.43;

EXPLANATION OF PLATE II

FIGURES 17–25: *Sinella alata* (all ×800 unless otherwise stated). FIG. 17.—External labial seta and same papilla (paratype). FIG. 18.—Internal setae of tibiotarsus (paratype), showing difference in ciliation. FIG. 19.—Male genital plate (holotype). FIG. 20.—Hind unguis (paratype), ×350. FIG. 21.—Middle unguis (another paratype), ×350. FIG. 22.—Mucro (holotype). FIG. 23.—"Sense" organ at apex of third antennal segment (left, pair from specimen from Shiloh Cave, Indiana; right, pair from paratype). FIG. 24.—Subapical pegs of fourth antennal segment (paratypes). FIG. 25.—Outline of antennal apex (holotype), showing position of subapical pegs. All magnifications are approximate.

FIGURES 26–31: *Sinella barri* (all ×800 unless otherwise stated; magnifications are approximate). FIG. 26.—Internal setae from tibiotarsus, showing ciliate and striate stypes (paratype). FIG. 27.—Hind unguis (specimen from Jesse James Cave, Tennessee), ×350. FIG. 28.—External seta of labial appendage, and same papilla (holotype). FIG. 29.—Basal half of male genital plate (specimen from Christian Cave, Tennessee). FIG. 30.—Mucro (paratype). FIG. 31.—Apical "sense" organs of third antennal segment (specimens from various localities).

FIGURES 32–40: *Sinella hoffmani* (approximate magnifications as indicated). FIG. 32.—Setae from internal surface of tibiotarsus, showing ciliate and striate types (specimen from Crossroads Cave, Virginia), ×800. FIG. 33.—Male genital plate (specimen from Perry Saltpeter Cave, Virginia), ×800. FIG. 34.—Hind unguis (topotype), ×350. FIG. 35.—Empodial appendage (another topotype), ×350. FIG. 36.—Hind unguis (specimen from Mystic Cave, West Virginia), ×350. FIG. 37.—Mucro (topotype), ×350. FIG. 38—Apex of fourth antennal segment (topotype), ×800. FIG. 39.—Apical "sense" organs of third antennal segment (right pair, topotypes; left pair, specimens from Crossroads Cave, Virginia), ×800. FIG. 40.—External seta of labial appendage and same papilla (topotype), ×800.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dens 0.52 (0.55); hind femur 0.26 (0.20); hind tibiotarsus 0.53; hind unguis 0.048; hind empodial appendage 0.038; hind tenent hair 0.027.

Type locality: Mays Cave, Monroe Co., Indiana (D. Burton, collector). Also known from Shiloh Cave, Lawrence Co., Indiana.

This species is known definitely from only two localities, both in central Indiana. The seven specimens in the series show little variation, most of that seen being in the small external tooth, which may be virtually invisible by being bent to one side. The largest basal tooth (in one specimen) was only one-third as long as the empodial appendage. A very short, median lamella may extend up a short distance from the basal teeth.

S. alata has probably been identified by some as *S. cavernarum*, which it does resemble in some respects. However, the peculiar pegs of the fourth antennal segment, the typical unguis, the character of the internal tibiotarsal setae, and especially the male genital plate, all serve to separate it from *cavernarum*. It is closely allied to *S. basidens*, but can readily be separated by the claw and antennal structure.

Sinella barri, new species

Figures 26–31

Facies typical for the genus; covered with small patches of reddish pigment except on venter and fifth and sixth abdominal segments. Head broadly oval. Antennae slightly more than twice as long as head, the segments subcylindrical, rounded lightly near apices; apex of third segment with "sense" organ of two paddle-like setae with entire margins and with supporting thickening along one margin or central; fourth segment without clearly differentiated sensory knobs or pegs; apex of third segment on ventral surface with six clearly distinguished, smooth, curved setae, these about one-third as long as neighboring ciliate setae. External differentiated seta of labial appendage exceeding apex of same papilla for about one-third its own length. Internal setae of tibiotarsus of two distinct types, the larger generally without ciliation but with fine striations on the surface, the other type with evident ciliation. Unguis with paired basal internal teeth starting just below its mid-level; a third, sharply acuminate, internal tooth midway between basal teeth and apex of unguis; and a very minute tooth just below the apex; all teeth on unguis relatively small. Tenent hair long, acuminate. Mucro typical of the genus. Male genital plate with slender setae, the basal one blunt, the others acuminate.

Measurements of holotype: Head, maximum 0.69; antennal segment 1–4, respectively, 0.19, 0.39 (0.37), 0.29 (0.36), 0.43 (0.52); second thoracic segment 0.34; third thoracic segment 0.27; abdominal segments 1–6, respectively, 0.16, 0.23, 0.22, 0.70, 0.16, 0.10; manubrium 0.52; dens 0.62; mucro 0.016; hind femur 0.42 (0.40); hind tibiotarsus 0.62 (0.60); hind unguis 0.072 (0.066); hind empodial appendage 0.033; hind tenent hair 0.036.

Type locality: Inman Cave, Perry Co., Tennessee (T. Barr, collector). This species is common in central Tennessee, and has been taken in central Kentucky.

The external seta of the labial appendage may extend beyond the apex of the same papilla for almost one-half, or for as little as one-fourth its length. Most commonly both apical organs of the third antennal segment are oval paddles, with the supporting thickening of one of them along one margin and that of the other in the middle; rarely the support may be bifurcate and the paddle triangular. The apical median internal tooth of the unguis usually is absent or is visible only under the highest phase-contrast magnification. The striate setae of the internal surface of the tibiotarsus are about as "smooth" as in any species of the genus.

This species is very distinct from all the other members of the group. In general structure it is closest to *S. avita*, but is easily distinguished from that species on the basis of the tibiotarsal setae. As regards the structure of these setae, *S. barri* is nearest to *S. hoffmani*, but the characters of the unguis and the labial appendage readily separate these two species.

Sinella hoffmani Wray

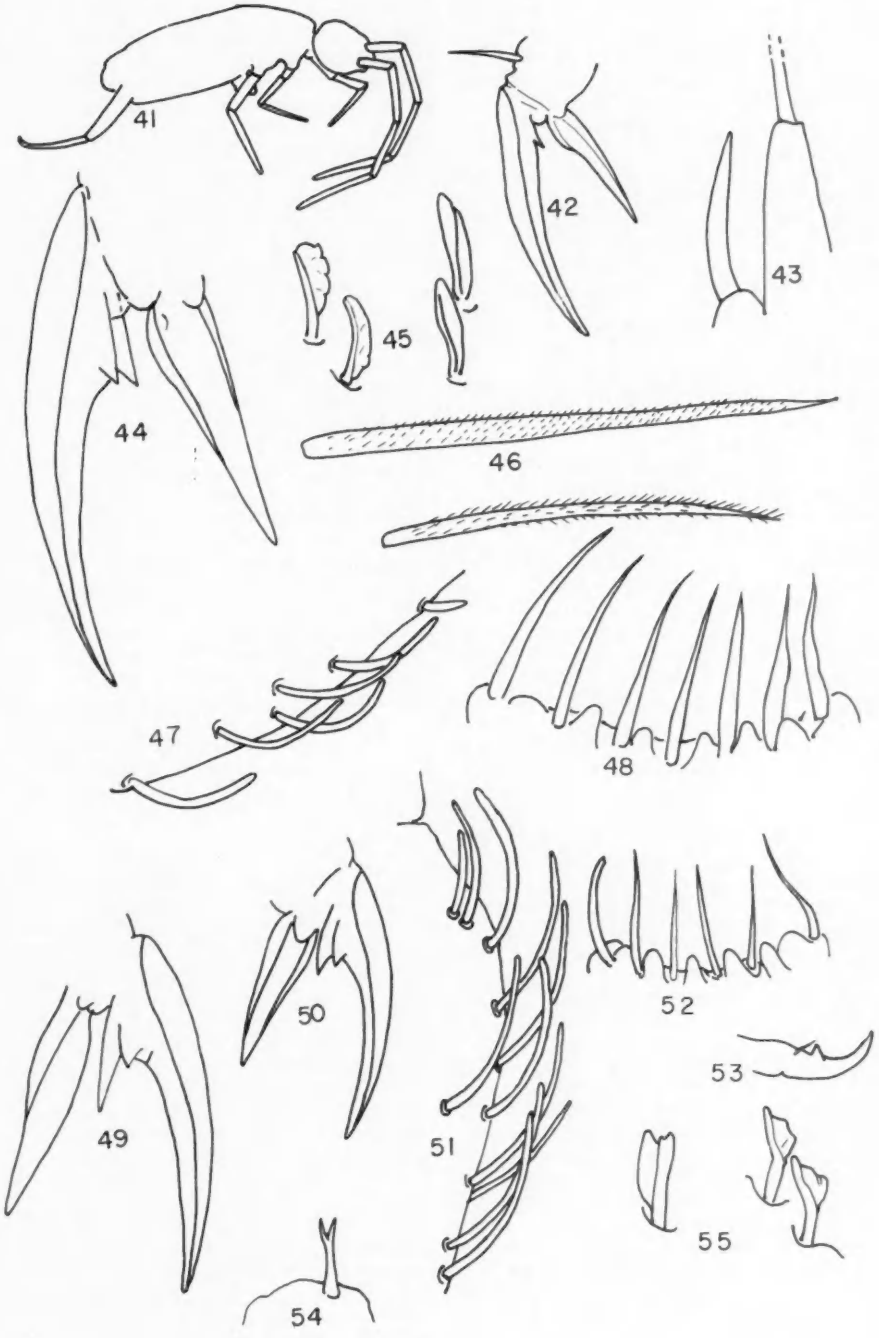
Figures 32–40

Sinella hoffmani Wray 1952: 95.

EXPLANATION OF PLATE III

FIGURES 41–48: *Sinella krekeleri* (all ×800 unless otherwise stated; magnifications are approximate). FIG. 41.—Habitus (paratype), ×12. FIG. 42.—Hind unguis, typical (paratype), ×350. FIG. 43.—External seta of labial appendage, and same papilla (paratype). FIG. 44.—Hind unguis, atypical (paratype). FIG. 45.—Apical "sense" organs of third antennal segment (right pair, holotype; left pair, paratype). FIG. 46.—Setae from internal surface of tibiotarsus (holotype), showing distinction between finely and coarsely ciliate types. FIG. 47.—Curved setae from apex of third antennal segment (paratype). FIG. 48.—Male genital plate (holotype).

FIGURES 49–55: *Sinella basidens* Bonet (all ×800 unless otherwise stated; magnifications are approximate). FIG. 49.—Hind unguis. FIG. 50.—Hind unguis (specimen from Avant Cave, Tennessee), ×350. FIG. 51.—Curved setae on apex of third antennal segment (specimen from small cave in Mercer Co., Kentucky). FIG. 52.—Male genital plate (specimen from same cave as preceding). FIG. 53.—Mucro (specimen from Avant Cave, Tennessee. FIG. 54.—Apex of fourth antennal segment (specimen from small cave in Mercer Co., Kentucky). FIG. 55.—"Sense" organs on apex of third antennal segment.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Facies typical of the genus; white, with faint reddish color on thorax and first four abdominal terga. Head oval. Antennae slightly more than twice as long as head; first three segments subcylindrical, fourth segment ellipsoidal; apical "sense" organ of third segment with two paddle-shaped pegs having supporting thickening along one margin, and the other margin serrate, other side of apex with 25 curved, blunt setae only poorly distinguished from the normal ciliate setae nearby and about one-third as long as these; fourth segment with a single oval, subapical peg and with unusually long ciliate setae, those in the middle region about as long as the segment is wide. Labial appendage with external differentiated seta just attaining apex of same papilla. Internal setae of tibiotarsus plainly differentiated into smaller, plainly ciliate, and larger, finely striated types. Tenent hair long, slender, acuminate. Unguis without lateral, external, or unpaired internal teeth; paired basal internal teeth large, the largest about as long as empodial appendage. Empodial appendage acuminate, with a minute tooth on external margin. Mucro typical for the group. Male genital plate with basal setae slightly blunted and somewhat larger than the others, which are narrow, acuminate, and simple.

Type locality: Lowmoor Quarry Cave, Allegheny Co., Virginia. Known generally from western Virginia and eastern West Virginia.

Specimens have been examined from a large number of localities, but all of the known records are from Virginia and West Virginia. These showed little variation, except in the structure of the "sense" organ of the third antennal segment, which often was triangular, as in *S. basidens*, with a bifurcate supporting thickening. The subapical peg of the fourth antennal segment was seen in only four specimens. In one population, from Star Chapel Cave, Bath Co., Virginia, the unguis showed an evident, median, unpaired tooth about half-way up on the internal edge, and in many specimens from various localities a small lamella was seen projecting a short distance beyond the basal teeth.

Sinella krekeleri, new species

Figures 41–48

Facies more elongate than is typical of the genus; white, without trace of pigment. Antennae more than three times as long as head, all segments subcylindrical; apical "sense" organ of third segment of two paddle-shaped pegs with supporting thickening along one margin; fourth segment without clearly differentiated pegs or clubs. Labial papillae with differentiated external seta barely surpassing apex of same papilla, and slightly thicker than apical seta. All internal setae of tibiotarsus unusually slender and finely ciliated, ciliations on larger setae usually smaller than those on smaller setae. Unguis unusually long, slender and sickle-shaped, without external teeth and with only a single pair of small, basal, internal teeth. Empodial appendage expanded basally, and slightly concave on internal edge of apical two-thirds. Male genital plate with all setae acuminate. Mucro typical of the group.

Measurements of holotype: Head, maximum 0.65; antennal segments 1–4, respectively, 0.33 (0.40), 0.53, 0.57, 0.88; second thoracic segment 0.31; third thoracic segment 0.23; abdominal segments 1–6, respectively, 0.12, 0.24, 0.18, 0.84, 0.20, 0.12; manubrium 0.51; dens 0.78; mucro 0.027; hind femur 0.65; hind tibiotarsus 0.70 (0.75); hind unguis 0.057; hind empodial appendage 0.034 (0.036); hind tenent hair 0.014 (0.015).

Type locality: Wind Cave, Jackson Co., Kentucky (C. Krekeler, collector). Common in caves of east-central Kentucky; one record from central Tennessee and one from northern Alabama.

In its typical form this is the most distinct member of the genus in the whole Nearctic Region. The small basal teeth and elongate unguis, together with the peculiar empodial appendages, serve to separate it from all other known *Sinella*. However, a good deal of variation was seen in the structure of the unguis, which, in a few specimens, became very like that found in *S. basidens*. The antennal ratios serve readily to distinguish *S. krekeleri* from that species.

Sinella basidens Bonet, new status

Figures 49–55

E[ntomobrya]. (Parasinella) cavernarum var. *basidens* Bonet 1931: 276 and 383 (nomen nudum); Bonet 1934: 368 (valid).

Facies typical of the genus. Head broadly oval. Antennae about 2¼ times as long as head, all segments subcylindrical; apex of third segment with "sense" organs consisting of two flat, triangular paddles supported by bifurcate thickenings, other side of apex with a prominent clump of 14 curved, blunt setae about one-third as long as neighboring cilate setae; fourth segment without clearly differentiated pegs or clubs. Differentiated seta of labial appendage barely surpassing apex of same papilla, and about as wide as apical seta. Internal setae of tibiotarsus prominent, moderately ciliate, the larger setae plainly more finely ciliate than the smaller ones. Tenent hair acuminate, slender. Unguis slender, sickle-shaped, without external, lateral, or unpaired internal teeth; one of the paired basal teeth about as long as unguis is wide, the other one small. Empodial appendage slender, acuminate. Male genital plate with all setae acuminite, the basal one somewhat thicker than the others. Mucro normally with anteapical tooth very small, less than one-half as long as apical tooth.

Measurements: Head, maximum, 0.74; antennal segments 1–4, respectively, 0.29, 0.46, 0.46, 0.60; abdominal segments 2–6, respectively, 0.21, 0.29, 0.88, 0.26, 0.13; hind femur 0.48; hind tibiotarsus 0.82; hind unguis 0.099; hind empodial appendage 0.057; hind tenent hair 0.039; manubrium 0.52; dens 0.74; mucro 0.030.

I recently examined one of the specimens described by Bonet as *S. cavernarum* var. *basidens*, and found it identical in most respects with the other specimens I have placed here. Bonet's type material was collected in Reid's Cave, Fayette Co., Kentucky, August 20, 1928, by Bob Jeann. *S. basidens* is now known from eastern and central Kentucky, and the material examined provides one record from central Tennessee.

This species seems closely related to *S. krekeleri*. Typical forms of the two species may readily be separated by the unguis and the empodial appendages, but individuals occurred in several populations which were difficult to separate by these characters; and, interestingly, the cases of approach in ungual structure occurred only in the allopatric parts of the ranges. The ratio of antennal length to head size remained clearly different in the two species, throughout their ranges.

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DETERMINATION OF NUCLEIC ACIDS IN THE TESTES OF THREE COCKROACH SPECIES[1]

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ABSTRACT

Nauphoeta cinerea Oliv., *Leucophaea maderae* (Fabr.), and *Blaberus craniifer* (Burm.) were the species used in this comparative study of nucleic acid content, and with *N. cinerea* three analytical methods were compared. Although the results obtained by these methods were variable, ratios of PNA and DNA tended to remain constant in last-instar nymphs and adults. This, and the fact that the weights of the organs were the same, indicated that the testes in the three species attained full growth in the last nymphal instar. Treatment of last-instar nymphs of *N. cinerea* with aminopterin showed no apparent effects on nucleic acid synthesis or on testicular growth.

Although the nucleic acids have been studied extensively for two decades, few biochemicals studies with these acids have dealt with insects. Nucleic acid content in whole insects was determined by Niemierko et al. (1956) and Heller and Jezewska (1958), who studied the silkworm and the moth *Antheraea pernyi*, respectively. Levenbook et al. (1958) isolated ribonucleic acid from *Drosophila* eggs and determined its constitution. Among the few workers who have studied individual insect tissues or organs are Patterson and Dackerman (1952), who determined the nucleic acid content in the salivary glands of *Drosophila* larvae, and Shigematsu and Takeshita (1958), who worked on silkworm fat body.

A study was undertaken at Beltsville, Md., to determine the nucleic acid content of the testes in last-instar nymphs and adults of three cockroach species, and to compare several methods of analysis in a single species.

MATERIALS AND METHODS

The deoxyribonucleic acid (DNA) standard (California Foundation for Biochemical Research) was from salmon sperm analyzing 8.1% phosphorus by the King (1932) method. The pentose nucleic acid (PNA) was from a sample of yeast nucleic acid that had been purified by the method

[1]Accepted for publication December 7, 1959.

of Kunitz (1940) and analyzed 9.1% phosphorus by the King method.

The three species of cockroaches were *Nauphoeta cinerea* (Oliv.), *Leucophaea maderae* (Fabr.), and *Blaberus craniifer* (Burm.). Male cockroaches, last nymphal instars and adults, were chosen randomly from the laboratory colonies. The testes were excised, weighed individually, and frozen immediately in dry ice to inhibit enzymatic activity. Groups of testes with wet weights of 30 to 65 mg. were used. The number of groups depended upon the amount of biological material available, which was limited for *Leucophaea* and *Blaberus*. The average weights per testis in milligrams with their standard deviations were: *Nauphoeta* adults 2.24 ± 0.66, nymphs 2.05 ± 0.34; *Leucophaea* adults 2.89 ± 0.35, nymphs 3.04 ± 0.31; and *Blaberus* adults 4.9, nymphs 6.02. No standard deviation was calculated for *Blaberus*, since the testes were pooled and weighed.

Schneider's method (1945) of extraction of the nucleic acids was used for comparison among species. In this method both PNA and DNA were in a pooled 5% trichloracetic acid extract, and the measurements were made on the sugar components of the nucleotides. Extractions were made on three separate groups of both nymphal and adult *Nauphoeta* and *Leucophaea*, but only a single group of *Blaberus* nymphs and adults was used because of the limited quantity of insects.

For PNA analysis the Mejbaum method (1939) for pentose sugars was used, and for DNA analysis the Dische method (1930) for deoxypentose sugars as modified by Patterson and Dackerman (1952). Since final amounts of the extracts were small (3 ml.), 0.5-ml. aliquots were used for the former and 0.3-ml. for the latter. All analyses were duplicated.

Absorption was determined on a Beckman spectrophotometer Model DU, at 670 mμ for PNA; dichromatic readings (600–650 mμ) were made for DNA as recommended by Dische (1955).

For the comparison of methods the testes of *Nauphoeta cinerea* were used. The nucleic acids were extracted by the Schneider (1946) adaptation of the Schmidt and Thannhauser (1945) method. The acids were separated from an alkaline hydrolysate of the tissues by precipitating the DNA with 6 *N* hydrochloric acid, leaving the PNA in solution. The DNA was then brought into solution by heating with 0.5 *N* perchloric acid, which has the advantage of low absorptivity in ultraviolet light.

The nucleic acids were estimated by measuring the sugar, the base, and the phosphorus components of the molecule. The sugar moieties were measured by the methods referred to above, the purines and pyrimidines by absorption in the ultraviolet region at 260 mμ, and the phosphorus by the following modifications of the method of Chen et al. (1956), since preliminary tests showed that the ashing procedure was not suited to these analyses:

The extracts (0.5-ml. aliquots) were digested with 0.6 ml. of 70–72% perchloric acid (A.C.S.) in blood-sugar tubes. These tubes were placed in a micro-Kjeldahl apparatus over a low gas flame to obviate bumping, and heated until thick white fumes appeared. They were then cooled and about 6 ml. of distilled water was added to each. One ml. of 2.5% ammonium molybdate (A.C.S.) and then 1 ml. of freshly prepared 10% L-ascorbic acid (A.C.S.) were added, and the mixture was diluted to 10 ml. with water. The tubes were placed in a water bath for 2 hours at 37° C., and absorption was measured in 1-cm. Pyrex cells in a spectrophotometer at 820 mμ against distilled water. Reagent blank values were substracted from the readings.

As in the original method, these steps place the phosphomolybdate reduction products well within the required range of acidity. The sensitivity of the original method was reduced by approximately one-half, but was more than ample for these investigations. This method was four times as sensitive as the Fiske-Subbarrow method (1925).

Since the folic acid analog aminopterin (4-aminopteroyl-glutamic acid) was found to be a potent inhibitor of ovarian growth (Mitlin et al. 1957), as well as larval growth (Mitlin et al. 1954) in the house fly, an experiment was designed to determine whether ecdysis could be prevented in the male cockroach, and also to test whether aminopterin would inhibit nucleic acid synthesis in testicular tissues as it has in other tissues (Brown and Roll 1955).

Thirty-five last-instar male *Nauphoeta* nymphs were segregated and placed on their regular diet of dog food (Ralston Purina). The water fed to them contained 0.1 mg. of aminopterin per milliliter as the sodium salt. This concentration will inhibit ovarian growth in the house fly (Mitlin, unpublished data). Twelve days later eight roaches had become adults. They were castrated and the nucleic acids extracted from the testes by the Schneider method (1945), and the sugar components were analysed. The remaining roaches were held to determine whether the chemical treatment would prevent ecdysis.

RESULTS AND EVALUATION OF DATA[2]

All results are reported in terms of micrograms of nucleic acid per mg. wet weight of testes; phosphorus determinations are based on the percent of phosphorus in the standard.

The amounts of nucleic acids in the two stages of the three species of roaches are given in table 1. By the "t" test, no significant differences ($P = 0.05$) between nymphs and adults could be shown

[2]Edwin L. Cox and James E. Koch of the Biometrical Services, Agricultural Research Service, U.S.D.A., assisted with the statistical analyses.

in either PNA or DNA content. Since there was only a single determination for *Blaberus*, this procedure could not be used, but the same appears to be true for that species. In addition, an analysis of variance of the combined data showed no significant differences between instars.

TABLE 1.—Nucleic acids in testes of nymphs and adults of three roach species based on sugar analyses (μg./mg. wet weight plus standard error of the mean)

Species and Stage	PNA	DNA	PNA/DNA
Nauphoeta			
Nymphs	4.2±0.3	3.9±0.3	1.08
Adults	5.0±0.7	4.6±0.1	1.09
Leucophaea			
Nymphs	3.2±0.2	2.8±0.2	1.14
Adults	3.2±0.5	2.8±0.5	1.14
Blaberus			
Nymphs	3.9	3.3	1.18
Adults	3.4	3.0	1.13

It was also shown that there was more PNA per mg. of testes for *Nauphoeta* nymphs than for *Leucophaea* nymphs. The difference approached the borderline of significance for adults. For DNA the situation was reversed; a significant difference was found for adults and a borderline value for nymphs. The values for *Blaberus* were between the other two.

All *Nauphoeta* nymphs fed aminopterin became adults. Analysis of the nucleic acid extracts of the testes gave 5.1 μg./mg. for PNA and 5.0 for DNA. These values were higher than those of untreated roaches but not significantly so. The ratio of 1.02 did not differ significantly from that of the untreated roaches.

The results of comparison of analytical methods using *Nauphoeta* are shown in table 2. These data were subjected to analysis of variance and significance was determined by Duncan's multiple range test (1955).

For the PNA determination in each of the nymphal and adult stages, the sugar and UV determinations appeared to show no significant differences at the 5% level. Phosphorus determinations were significantly higher in both stages. DNA followed a different pattern; sugar and phosophorus determinations were not significantly different, but the UV determinations were significantly lower.

A combined analysis of variance for each aci.. showed that for PNA the difference between nymph and adult approached significance at the 5% level and was significant at the 10% level. For DNA the difference was significant at the 5% level. Nor were PNA/DNA ratios uniform. They ranged from 0.64 to 1.35 by measurement of the different components.

DISCUSSION

Evidently both extraction and analytical methods leave much to be desired when applied to cockroach testes. The values obtained by the sugar analyses, using both Schneider's method and his adaptation of the Schmidt and Thannhauser method, agree within error for PNA in both *Nauphoeta* nymphs and adults, but this does not hold true for DNA. The differences, particularly for DNA, found by measuring the base and phosphorus components, cannot be reconciled.

Such variations have been found with other tissues (Webb and Levy 1958). The standards may well be a contributing factor, since they are derived from tissues rather widely removed from those of insects. As Levenbook et al. (1958) pointed out, different fractions of cell homogenates may contain nucleic acids of different base compositions. They found it necessary to prepare pure standards from *Drosophila melanogaster* Meig. in work with eggs from that insect.

In spite of the lack of agreement in the values found for content by the different methods of extraction and analysis, the ratios of PNA to DNA remained essentially the same for both nymphs and adults. If the PNA/DNA ratio is used as a measurement of growth, then perhaps growth of the testes is complete at the last nymphal instar.

TABLE 2.—Comparison of nucleic acid content of testes of nymphs and of adults extracted by the Schmidt and Thannhauser method (1945) as modified by Schneider (1946). Means of three experiments in μg./mg. of wet weight.

Analysis and Stage	PNA	DNA	PNA/DNA
Sugar			
Nymphs	3.5[a]	5.5[a]	0.64
Adults	5.4[a]	8.5[a]	0.64
Phosphorus[b]			
Nymphs	6.4	7.2[a]	0.89
Adults	8.4	8.5[a]	0.99
Ultraviolet			
Nymphs	4.2[a]	3.1	1.35
Adults	5.6[a]	4.3	1.30

[a]Means not significantly different for nucleic acid within age group.
[b]Based on percent of phosphorus in the standard.

Such a condition is not unusual among insects. Qadri (1938) reported that the testes of *Blatta orientalis* L. are functional at the end of nymphal life, and in *Drosophila melanogaster* Meig., which with a complete metamorphosis, is much higher on the evolutionary scale, the testes are full grown and contain mature spermatozoa before eclosion (Bodenstein 1950).

The results of the aminopterin experiment are inconclusive. There was no significant difference

in weights or nucleic acids of the testes of nymphs and adult *Nauphoeta*. But a conclusion based on the amounts of nucleic acid per unit weight of tissue can be unreliable, since changes could arise from alteration of other cellular constituents (Brown and Roll 1955).

To obtain more reliable information, the nucleic acids of such organisms should be examined by analysis of the constituent purine and pyrimidine bases.

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A NEW SPECIES OF OMANIA FROM SAMOA, WITH NOTES ON ITS BIOLOGY (HETEROPTERA: SALDIDAE)[1]

W. R. KELLEN[2]

ABSTRACT

Omania is a genus of little-known marine saldids which inhabit coral and volcanic rocks in the intertidal zone, and previously included two species, from Arabia and from the Great Barrier Reef, Australia, respectively. The present species is abundant in Samoa, and was easily reared under laboratory conditions to provide the data presented here on life history and behavior. Presumably other *Omania* species occur on other islands in the South Pacific.

The genus *Omania* was erected by Horváth in 1915 to accommodate the species *O. coleoptrata* described from a unique female specimen collected by L. Biró in 1902. This single individual was found in a mass of marine algae in the intertidal zone at Masqat (Muscat) in the province of Oman, Arabia. Subsequently a unique male specimen of this same species was collected by R. Dollfus in 1928 on the Island of Senafir at the southern extremity of the Sinai Peninsula. The male individual was found in a rock crevice below the high tide level of the intertidal zone. A second species belonging to the genus was recently described by Woodward (1958) from a long series of specimens collected by E. N. Marks

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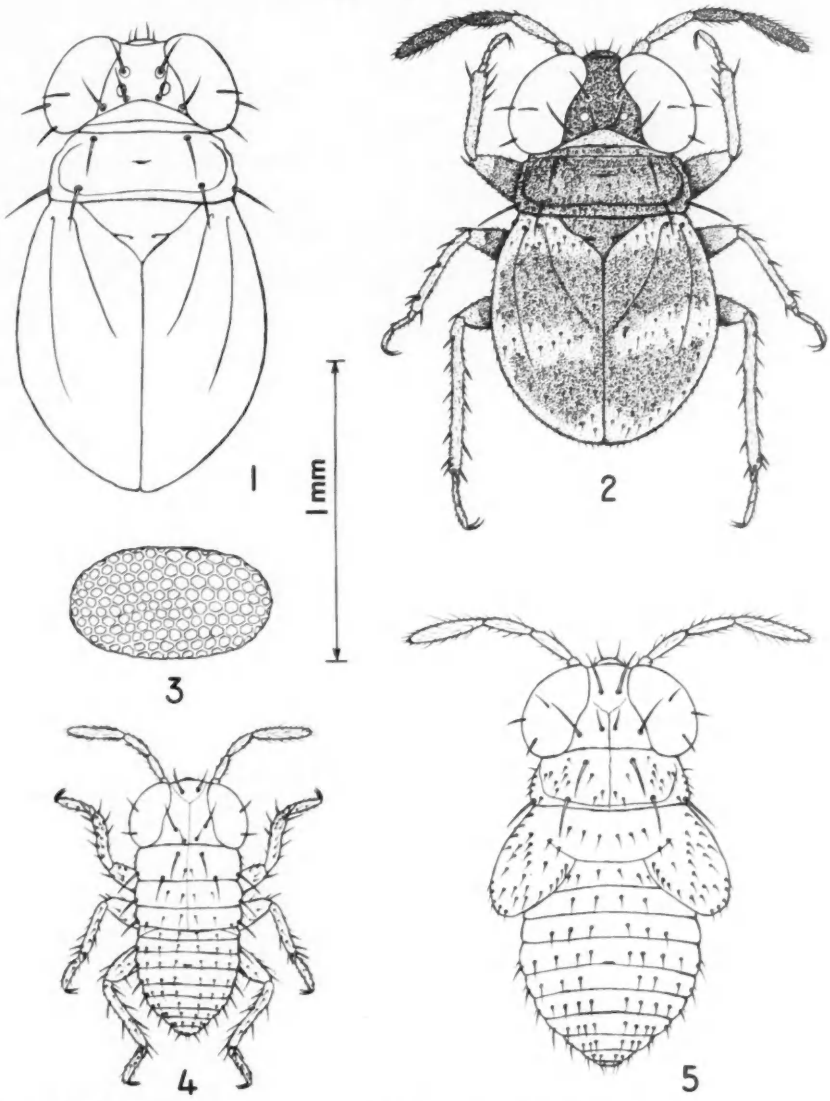

FIG. 1.—Dorsal view of female. FIG. 2.—Dorsal view of male. FIG. 3.—Egg. FIG. 4.—Dorsal view of first instar nymph. FIG. 5.—Dorsal view of fifth instar nymph.

at the Low Isles and by Woodward at the Heron Isles in the Great Barrier Reef, Australia; this species, *O. marksae*, was also found in the intertidal zone. Woodward collected all the nymphal instars, together with adults apparently living in small holes in coral rocks exposed at low tide. Woodward's excellent paper describes each instar and discusses the adaptations of the saldid to marine life.

The present species is the third member of the genus to be described, and the first recorded from Oceania. It seems very likely, however, that many more members of this interesting genus will be found in the tropical and semitropical marine beaches from Africa to Oceania, having eluded discovery thus far by their minute size and secretive nature.

Omania samoensis, new species

MALE. Small, brachypterous, coleopterine (fig. 2). Vertex, frons, and clypeus shiny black, punctate, with abundant, short, decumbent, stramine-

ous setae; the four erect, black bristles on vertex prominent; preocellar bristles on prominent tubercles (fig. 9). Rostrum reaching to mesocoxae, testaceous. Collum slightly swollen, with dull silvery pruinosity, anterior margin broadly arcuate. Eyes large, dark red, the three macrotrichiae on each black. Antennae with abundant, stramineous, fine setae; first two segments stramineous, the last two fuscous.

Pronotum, scutellum, and hemelytra with sparse, short, stramineous, posteriorly directed setae. Pronotum dull black, the disk swollen; collar distinct; anterior margin with two, strong, erect black bristles, another pair on posterior margin before prominent carina; posterior lateral angles of pronotum also with a pair of strong bristles (fig. 8). Mesothorax fused to metathorax (fig. 11); metapleura broadly produced posteriorly to shield hind coxal cavities.

Hemelytra entirely coriaceous, strongly convex; claval suture and radius present as shallow grooves; color dull black with irregular silvery-blue patterns on basal, middle, and apical areas, extending along anterior margin. Pleural area dull, pale, fuscous, glabrous; spiracles and sclerites distinct.

Abdomen fuscous with abundant, short, stamineous, setae ventrally; tergites weakly sclerotized (fig. 6). Genital capsule not prominent, with a pair of minute claspers (figs. 12, 14, 15).

Coxae and most of femora shiny testaceous; apical fifth of femora, tibiae, and tarsi shiny stramineous with abundant setae. Claws simple.

Total length, 1.4 mm.; width across middle of hemelytra, 0.7 mm.; ratio of antennal segments (1–4), 8:11:9:16; pronotum more than twice as wide at base as long, 40:18; head twice as long as interocular width at level of ocelli, 30:16; head width across eyes about three times greater than interocular width, 44:16; femora of first two legs subequal, the third longer, 25:26:31; tibiae of first two legs subequal, the third longer, 22:24:38.

FEMALE. Similar to male, but larger. Total length, 1.6 mm.; width across hemelytra, 0.8 mm. (figs. 1, 7, 13).

Holotype male, allotype female, I–10–58; 4 paratypes, IV–28–58; 15 paratypes, V–2–58; 5 paratypes, IX–8–58; all collected by the author at Tafuna, Tutuila Island, Samoa, and deposited at the U. S. National Museum, Washington, D.C.; 10 paratypes, V–2–58, deposited at the Bishop Museum, Honolulu, Hawaii. All of above are mounted on pins. Twenty-six paratypes, 67 nymphs, and 6 eggs mounted on slides also deposited at the U. S. National Museum.

The present species can be distinguished from *O. marksae* Woodward by the following characteristics: the irregular light areas on the hemelytra are much bluer in *marksae* and more silvery in *samoensis*; the fine setae on the head of *marksae* are silvery, while in the present species they are golden; the second antennal segment of *marksae* is infuscated on the apical half, while the same segment of the present species is entirely stramineous.

O. samoansis can be distinguished from *O. coleoptrata* Horváth by the relatively smaller second antennal segment; in the male of *coleoptrata* the relative lengths of the segments (1–4) are 9:13:8.14 (from China 1938), while in the present species they are (1–4), 8:11:9:16; moreover, the third segment and basal half of the fourth are blackish in *coleoptrata*, while these same segments in *samoensis* are uniformly dark brown.

BIOLOGY

In Samoa a fairly long series of these small saldids were collected by repeatedly searching volcanic rocks lying in the intertidal zone of a shallow, protected lagoon. About 3 feet of tide were experienced in the lagoon, exposing about 25 feet of beach at low tide. There were no coral formations in the immediate vicinity; however, the surrounding shore area was characterized by an abundance of volcanic rocks about a foot in diameter, a cinder beach, and much silt from rain runoff. The saldid adults and nymphs were found frequenting small holes and crevices in the volcanic rocks. Interestingly, only those rocks lying in a rather narrow middle band of the intertidal zone were inhabited; apparently the acceptability of a particular rock is greatly influenced by the moisture conditions obtained in the pores when they are exposed to the sun at low tide. Those rocks lying at the broad extremes of the intertidal zone presumably are either too dry or too wet for habitation.

At high tide the rocks inhabited by the bugs are covered by as much as 2 feet of water; during this period the saldids are apparently safely protected in air bubbles trapped in the interstices of the rocks. Examination of the rocks at low tide when the saldids are active indicated that the bugs mainly restrict their movements to the lower portions of rocks kept moist by close proximity with the beach, where they are protected from the heating and drying effects of the sun. After overturning rocks, much patient waiting and very close examination were required to find the minute saldids as they crawled out from their hiding places. The bugs ran rather rapidly over the rock surface, but they usually did not travel very far, preferring to move from one small hole or crevice to another, probing the surface of the rock and small growths of algae with their beaks. Because of the irregularities of the rocks the little bugs were very difficult to capture, particularly as they quickly retreated to a hole if even slightly disturbed; moreover, their jumping ability is highly developed.

Although the saldids are apparently present and active in all months of the year, they appeared to be much more abundant during the

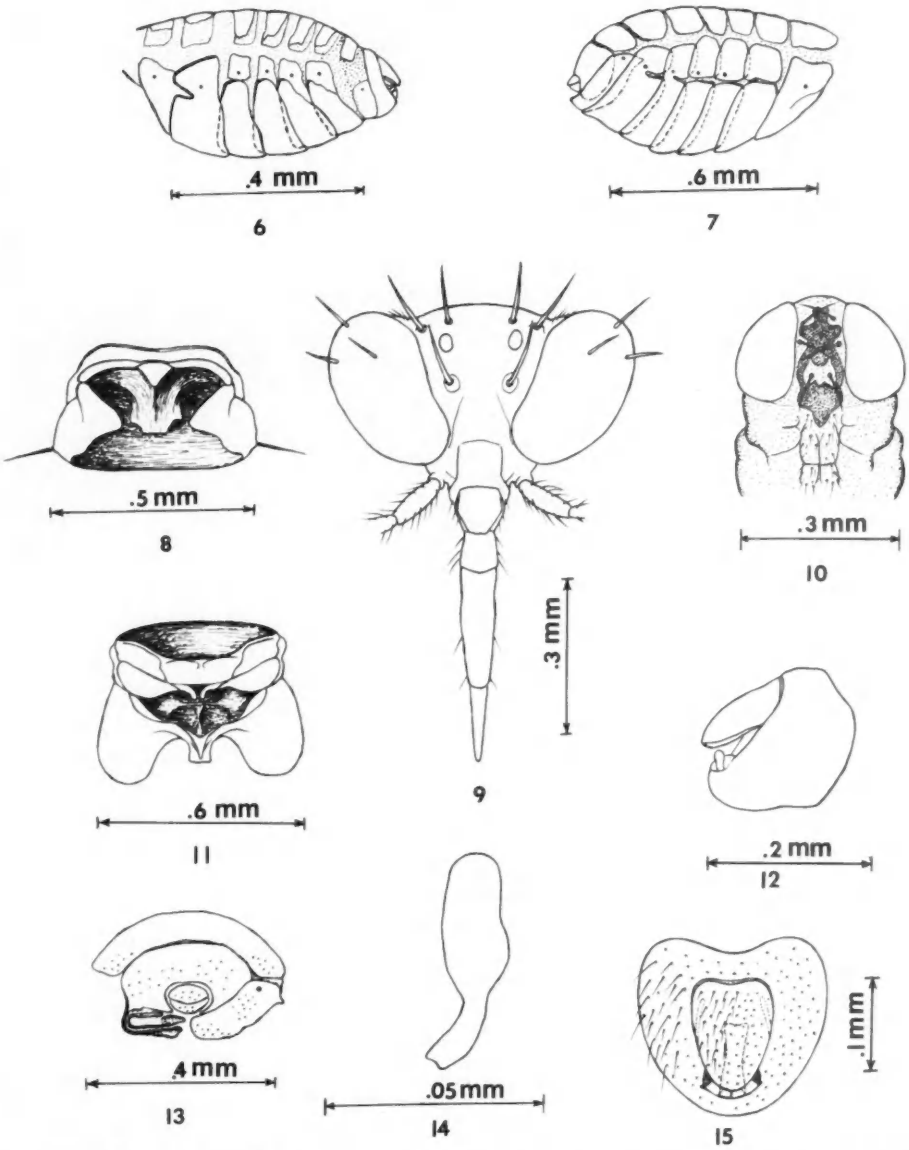

FIG. 6.—Lateral view of male abdomen. FIG. 7.—Lateral view of female abdomen. FIG. 8.—Ventral view of male prothorax. FIG. 9.—Anterior view of male head. FIG. 10.—Anterior portion of 15 day old embryo. FIG. 11.—Ventral view of male meso-metathorax. FIG. 12. Lateral view of male terminalia. FIG. 13. Posterior view of female terminalia with left sternite removed. FIG. 14.—Male clasper. FIG. 15.—Dorsal view of male terminalia.

months from January to June. In the laboratory they were easily reared by confining them to small stender dishes with pieces of wet algae collected from the natural habitat; small pieces of rock were also supplied to provide the bugs with natural hiding places. Individuals reared under these conditions survived very well and laid eggs, and new generations of nymphs were also reared to the adult stage without difficulty. Besides the organisms present in the algae, small psychodid and tendipedid larvae were provided as food.

In contrast with the timid behavior of the bugs in their natural habitat, they appeared to be highly attracted to moving objects under laboratory conditions. Instead of running to a position of concealment, the bugs stopped and turned toward a moving object, as if drawn by curiosity. This type of behavior was also expressed in the manner in which one individual reacted to another whenever they happened to meet. Almost invariably they approached to within a few millimeters of each other, and after hesitating a moment one individual suddenly jumped onto the back of the other. This apparently took place without regard to the sexes of the individuals involved, and in the great majority of instances no attempt at copulation was made. Possibly this is a type of recognition pattern, and perhaps when the proper stimuli are present the activity leads to copulation.

Eggs were not found in the natural habitat, although several rocks were broken open and searched. In the laboratory, however, eggs were laid deep in small holes and crevices in the rocks provided. The eggs are very large relative to the size of the female, and apparently only one egg is laid every few days (fig. 3). The average incubation period of seven eggs observed in the laboratory was about 16 days at an ambient temperature of 25° C. The eggs were creamy white when first laid, and the surface sculpturing was very distinct. By the fourth day the eyes were first visible as dark yellow areas, while the rest of the egg was light yellow. The eyes became light pink by the seventh day, and the rest of the egg appeared yellow-orange due to the color of the developing embryo. By the 10th day the egg was light red-orange, and by the time the nymph was ready to hatch the egg had developed a dark orange appearance, while the eyes appeared distinctly deep red and the egg burster was faintly visible (fig. 10).

Seven nymphs reared individually from eggs to adulthood required the following times for development at 25° C.: first stadium, 4–6 days; second stadium, 4–6 days; third stadium, 4–8 days; fourth stadium, 4–8 days; fifth stadium, 6–10 days. The first- and fifth-instar nymphs are illustrated in figures 4 and 5, respectively.

In order to gain information on the relative growth rates of the nymphs a number of selected measurements were made of each instar. These data are presented in table 1 along with comparable measurements of the adults. It was found that there was very little variation from one individual to the next so only the average measurements of five individuals are given in each instance.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Dr. T. E. Woodward, University of Queensland, Brisbane, Australia, for making available specimens of *O. marksae* for comparison with the present new species, and to Dr. A. R. Barr for reading the manuscript.

TABLE 1.—Selected measurements of nymphs and adults of *Omania samoensis* n. sp. Figures are given in millimeters and are the average measurements of five individuals.

Instar	1	2	3	4	5	Male	Female
Total length	0.90	0.96	1.14	1.31	1.42	1.44	1.59
Body length	0.65	0.70	0.87	0.97	1.08	1.04	1.17
Hemelytral length	——	——	——	——	——	0.81	1.00
Hemelytral width	——	——	——	——	——	0.39	0.50
Pronotal length	0.13	0.14	0.15	0.18	0.21	0.23	0.26
Pronotal width	0.38	0.38	0.42	0.49	0.52	0.54	0.59
Head length	0.25	0.26	0.27	0.34	0.34	0.40	0.42
Head width	0.42	0.42	0.47	0.52	0.56	0.60	0.62
Eye length	0.24	0.25	0.27	0.30	0.34	0.36	0.38
Ant. 1	0.07	0.07	0.08	0.10	0.11	0.11	0.12
Ant. 2	0.10	0.10	0.11	0.13	0.14	0.15	0.16
Ant. 3	0.08	0.08	0.10	0.11	0.13	0.13	0.14
Ant. 4	0.19	0.19	0.20	0.21	0.22	0.22	0.23
Distance between anterior pronotal bristles	0.14	0.16	0.17	0.18	0.20	0.22	0.23
Distance between outer post-ocellar bristles	0.15	0.16	0.16	0.18	0.19	0.21	0.22
Leg 1 Femur	0.22	0.25	0.25	0.29	0.32	0.35	0.39
Tibia	0.20	0.20	0.23	0.27	0.29	0.30	0.34
Tarsus	0.11	0.12	0.13	0.14	0.15	0.14	0.16
Leg 2 Femur	0.21	0.22	0.26	0.30	0.33	0.36	0.41
Tibia	0.19	0.21	0.24	0.28	0.30	0.33	0.35
Tarsus	0.11	0.13	0.13	0.14	0.16	0.16	0.16
Leg 3 Femur	0.25	0.26	0.30	0.35	0.39	0.42	0.48
Tibia	0.26	0.29	0.35	0.41	0.46	0.51	0.58
Tarsus	0.15	0.15	0.17	0.20	0.20	0.21	0.22

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ASSAY OF THE PRINCIPAL GUSTATORY STIMULANT FOR THE TOBACCO HORNWORM, PROTOPARCE SEXTA, FROM SOLANACEOUS PLANTS[1]

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ABSTRACT

This stimulant was isolated from tomato and related plants of the family Solanaceae. Preliminary characterizations indicate the material to be a glycosidic substance; however, its complete chemical identity has not been elucidated as yet. In the presence of this substance, the insect attacks filter paper, lettuce leaves or artificial agar diets, but only when certain nutrients, particularly sugars, are also present. Filter-paper assays were employed during the early and agar-diet assays during the later stages of purification. The material is also active as a feeding stimulant for the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), which feeds on plants of the same family.

Various workers have shown that specific plant substances extractable from the respective host plants of oligophagous insects regulate their feeding. Verschaffelt (1910) first reported that a substance, sinigrin, present in cruciferous plants, governed the feeding of the larvae of the cabbage butterflies *Pieris rapae* L. and *P. brassicae* L. Sinigrin, as well as two other mustard oil glycosides, glucocheirolin and sinalbin, were subsequently shown to influence similarly the feeding of the diamondback moth, *Plutella maculipennis* (Curt.), by Thorsteinson (1953). Dethier (1941) reported that several essential oils of umbelliferous plants govern the choice of food plants of *Papilio ajax* L. while an unidentified glycosidic substance extractable from potato leaves was shown to stimulate the feeding of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) by Chauvin (1945, 1952). These findings and others (reviewed by Fraenkel 1956, 1959) form the basis for the postulate that host-plant specificities of oligophagous insects are mediated by plant substances of limited distribution. These substances are variously designated as attractants, token stimuli, and phagostimulants when they serve in such capacities. As chemicals, they are generally classified as secondary plant substances since their functions in plants are largely unknown (Paech 1950).

In respect to the tobacco hornworm, *Protoparce sexta* (Johan), its specificity to solanaceous plants was previously suggested to be governed by substances common to this group of plants (Yamamoto and Fraenkel 1960). A purified material from solanaceous plants has since then been shown to stimulate the feeding of the hornworm in a manner similar to attractants for other host specific insects. The preparation and assay of this material therefore form the basis for the report given here.

MATERIALS AND METHODS OF ASSAY

Insects used in the assay of extracts from various plants were reared in the laboratory. Plants used in the preparation of the extracts were obtained from fields and dried at room temperature. Subsequently they were powdered in a ball mill. Powdered leaves of tomato formed the bulk of the materials used in the extraction of the attractant, while other solanaceous plants were employed on a smaller scale to verify the presence of similar feeding stimulants in these plants.

Crude and semipurified extracts were assayed principally on filter paper, a technique found useful because of its ease and rapidity. Whatman No. 1, 9.5 cm.-diameter filter papers were marked off with ½-inch semicircles on the edges and, depending upon the number of fractions of the extract to be tested at one time, two, three, or four semicircles were drawn on each paper with a compass (fig. 1). Two drops of a test fraction in solution were placed on an appropriate semicircle with a micropipette gauged to deliver approximately $\frac{1}{100}$ ml. per drop. Each paper,

[1]Portion of a dissertation submitted by the first-named author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology, University of Illinois. This work was supported by a research grant from the Rockefeller Foundation. Accepted for publication December 11, 1959

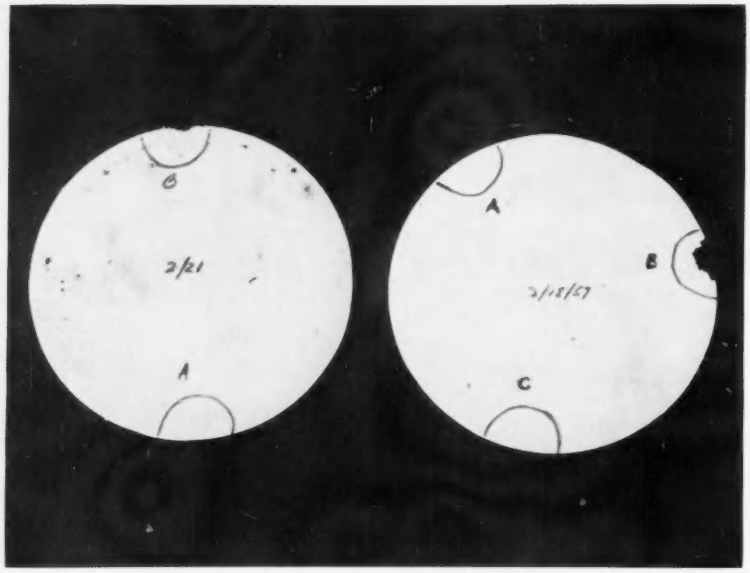

FIG. 1.—Examples of the types of biting responses obtained with 5th instar hornworm larvae exposed to stimulatory materials spotted on designated edges of circular filter papers. The larvae were exposed to the materials over a period of 2 hours.

after spotting with the test fractions, was placed in a 10-cm.-diameter battery jar and 1.0 ml. of water was then applied to the center of the paper. Diffusion of the water outward moistened the entire paper and thus prevented larval reactions to moisture at the points of application of the test fractions. One semicircle was always left blank as a negative control, and later, when biting responses to sugars were observed, a 0.1% solution of glucose was substituted in place of the blank. Generally 9 to 12 individual tests were run for each fraction of material obtained during purification. The larvae were not starved prior to the tests and each test was run for 2 hours. Positive responses consisted of biting or chewing of the filter paper at the point of application of the test material (fig. 1). A record of the responses was kept during the preparation and purification of the extract from the leaf powder.

The final purified preparation from tomato and other solanaceous plants was assayed on an agar-gel diet. The diet, based on preliminary feeding tests, consisted of the following per 25 ml. of water: agar, 1 gm.; cellulose, 1 gm.; casein, 0.5 gm.; yeast 0.125 gm.; and glucose, 0.45 gm. (0.1M.). In testing for the stimulatory effect of various sugars, yeast was eliminated from the diet since it contained sugars. The physical consistency of the diet was important in that soggy or soft diets were not as acceptable to the larvae as the firm diets afforded by using agar at the 4% level. Cellulose powder was included in the diet to give sufficient bulk to the fecal pellets; its presence did not affect the palatability of the diet. Casein was not stimulatory to feeding at the concentration used in the diet.

In preparing a diet, the basic components of the diet were mixed together with the extract of a plant in a beaker, heated to 80° C. in a water bath, and poured into petri dishes while hot. After the diet had cooled and hardened, 10-cm. discs were punched out with a clean cork borer. The discs were placed on 1-inch-square wax papers, and both discs and wax papers were then placed into petri dishes lined with filter papers. Each petri dish contained two discs of an experimental diet and two third-instar larvae. Six or more replicates were run for each experimental diet in the manner described above for 18 hours. The larvae became conditioned to the diets in about 2 to 4 hours and fed on them intermittently thereafter. The various diets were evaluated on the basis of the number of fecal pellets deposited over the time interval of the experiments.

Preparation of the Feeding Stimulant.—In an earlier work on the feeding behavior of the tobacco hornworm, Morgan and Crumb (1928) reported that either ether extracts or steam distillates of tobacco, *Nicotiana tabacum* L., and jimson weed, *Datura stramonium* L., elicited biting and feeding responses when the extracts or distillates were coated on nonhost plants or incorporated in paraffin wax. This work, how-

TABLE 1.—Biting responses of hornworm larvae to water-soluble substances from fresh leaves of various plants, spotted on filter paper. Duration of tests was 2 hours.

Plant	Number of replicates	Biting responses	
		Number	Intensity
Solanum nigrum (Solanaceae)	6	5	+++
Datura Stramonium "	6	4	+++
Lycopersicon esculentum "	10	10	+++
Morus sp. (Moraceae)	6	3	+
Convolvulus sp. (Convolvulaceae)	6	2	+
Quercus sp. (Fagaceae)	6	0	−
Catalpa sp. (Bignoniaceae)	9	3	++
Fraxinus sp. (Oleaceae)	9	0	−
Plantago sp. (Plantaginaceae)	9	3	+

+++ = Biting response intense and over large area.
++ = Biting response less intense.
+ = Biting response very light.
− = No response.

ever, was not confirmed when repeated in this laboratory. On the other hand, water or ethanol extracts of the residues from the ether extracts or steam distillates were highly attractive to the hornworm larvae. Subsequent extractions of tomato-leaf powder with several common organic solvents showed that the active material was soluble only in water, methanol, and dilute ethanol Hence leaf powders were extracted initially in hot 80% ethanol prior to further treatment.

Secondary plant substances present in solanaceous plants which were partially purified and assayed included the following: tropane alkaloids from *Datura*, nicotine and related compounds from tobacco, solanine from potato sprouts, and tomatine and flavanoid compounds from tomato leaves. The flavanoid compounds were tested since an unidentified flavanoid glycoside was implicated as the attractant for the Colorado potato beetle, an insect which also feeds on solanaceous plants. All of these materials, assayed on filter paper, were not attractive to the hornworm.

By elimination of some of the inactive secondary plant substances in the crude ethanol extract, an active fraction was obtained. The presence of a glycosidic substance in this fraction was indicated by tests which showed an increased reduction of Fehling's solution after treatment with dilute acids, inactivity of the extract after treatment with acids, and the uptake of the active substance on charcoal, a method used in the estimation of glycosidic compounds. The final preparation of the active material from crude extracts hence included procedures suggested for the isolation of glycosidic compounds (Trim 1956). The procedure involved yeast fermentation of the free sugars, lead acetate and magnesium oxide treatments and charcoal chromatography. The fraction eluted from a column of charcoal-celite (1:1) mixture with 10% pyridine proved to be the most active and free from common nutrient contaminants such as sugars and amino acids. The material is not readily crystallizable, very hygroscopic, colorless, and easily hydrolyzed with dilute acids to give hexose as one component, identified tentatively by paper chromatography and osazone formation. The material was assayed on filter paper and in agar diets. A detailed presentation of the preparation and purification of the active substance will be reported elsewhere after further chemical characterization of the substance has been completed.

TABLE 2.—Biting responses of the hornworm larvae to various nutrient materials spotted on filter paper over a period of 2 hours.

Nutrient	Number of larvae	Number of of larvae responding	Intensity of responses
B-vitamin mixture	12	5	++
Yeast, 2% solution	12	4	++
Glucose, 0.1 M	9	4	++
Sucrose, 0.1 M	12	7	++
Inositol, 0.1 M	12	6	++
Casein hydrolysate, 2% solution	9	2	+
Tomato leaf extract, 0.5% solution (control)	12	8	+++

Biting responses as given in table 1.

RESULTS AND DISCUSSION

Preliminary to the preparation of a purified extract, water extracts of various plants were tested for their stimulatory effect on the larvae

of the hornworm. Using the filter paper assay method (fig. 1), it was possible to discern that substances in the extracts of solanaceous plants only were highly stimulatory to feeding. The results of a test of this type, recorded on the basis of the intensity of biting and chewing of the filter paper, is given in table 1.

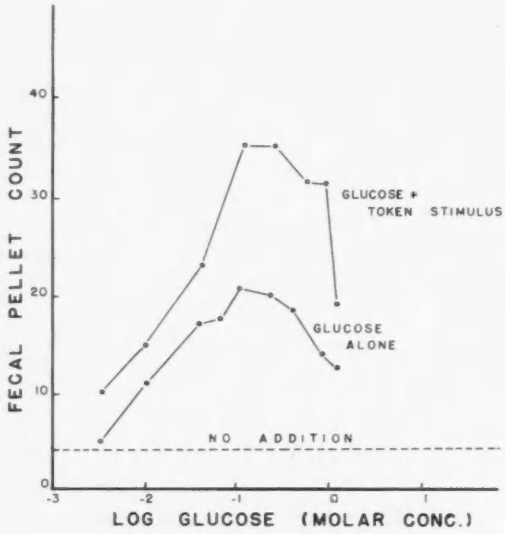

FIG. 2.—The effect of adding equal amounts of the purified token stimulus (0.64 mg. per gram of diet) to diets containing increasing concentrations of glucose on the fecal pellet count of hornworm larvae. Each point represents the average fecal count of 12-3rd instar larvae over a period of 18 hours. The basic components of the diets consisted of 1 gm. agar, 1 gm. cellulose, 0.5 gm. casein, and 25 ml. of water.

The stimulatory effect of certain nutrients, listed in table 2, was tested after it was noticed that responses to the plant extracts diminished with purification. Also during purification of the extract, a stimulatory substance was isolated in crystalline form which chemical analysis proved to be inositol, a rather common constituent of many biological materials. Hence in the cruder plant preparations, the combination of several substances afforded a highly stimulatory substrate whereas with purification and elimination of some of the substances, the preparation became less stimulating. The dependence of the activity of the stimulant to sugars, in particular, is best seen in the results obtained with agar diets. Maximal feeding on the agar diets was obtained at glucose concentrations between 0.1 and 0.4 M. Other sugars found in tomato leaves, namely sucrose, fructose, and inositol, gave similar response curves; however, the threshold for sucrose was much lower, the optimal concentrations being between 0.02 and 0.06 M. The response to diets containing glucose at various concentrations and also responses to similar diets containing a uniform amount (0.64 mg. per gram of diet) of the token stimulus is shown in figure 2. Figure 3 shows that a threshold level of the token stimulus (about 0.25 mg. per gram of diet in this test) is required to maintain maximal feeding. Materials prepared similarly from solanaceous plants in the genera *Nicandra*, *Petunia*, *Nicotiana*, *Solanum*, and *Physalis* also gave similar types of feeding responses on the agar diets.

The token stimulus for the tobacco hornworm was also tested on the Colorado potato beetle since this insect too feeds on solanaceous plants. The larvae of this insect do not feed on agar diets very well, hence the filter paper assay was employed. The material was spotted on filter paper in combination with 0.02 M. sucrose. Sucrose alone at the same concentration was used as control. The material, tested in this manner was highly attractive to the beetle. Whether the active material prepared by Chauvin (1945, 1952) from potato leaves and termed a flavone glycoside is the same substance prepared for the hornworm from tomato leaves has not been determined as yet.

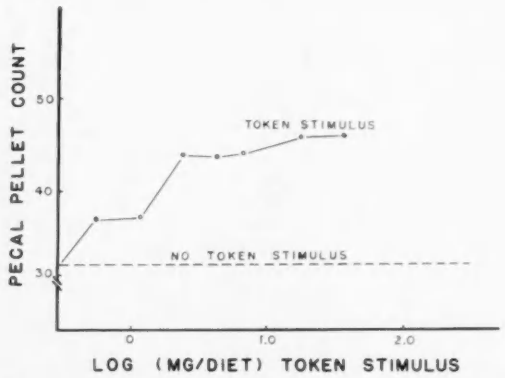

FIG. 3.—The effect of increasing amounts of purified token stimulus incorporated in agar-nutrient diets on the fecal pellet count of hornworm larvae. Each point represents the average fecal pellet count for 12-3rd instar larvae over a period of 18 hours. The diet consisted of the following materials: agar 1 gm.; cellulose 1 gm.; casein 0.5 gm.; yeast 0.125 gm.; glucose 0.450 gm.; and water 25 ml.

The finding that the stimulatory effect of the token stimulus for the hornworm is maximal in the presence of nutrients, in this case sugars, is essentially in agreement with the work of Thorsteinson (1953), who showed that sinigrin, a feeding stimulant for the diamondback moth, was most stimulatory in diets containing glucose, yeast, and pea-leaf powder. Yeasts or small amounts of tomato-leaf powder (0.5%) has also been observed to increase the feeding activity of the hornworm on the agar diets, however the

stimulatory effect of the token stimulus was not dependent upon their presence. The fact that feeding occurs on a nutrient or sugar fortified diet in the absence of the token stimulus (fig. 2) indicates that sufficient gustatory stimulation already exists. It would seem then that the role of the token stimulus is one of reinforcing the basal feeding on a diet by providing additional or maximal stimulation.

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THE SPECIFICITY OF THE TOBACCO HORNWORM, PROTOPARCE SEXTA, TO SOLANACEOUS PLANTS[1]

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ABSTRACT

Oviposition and feeding of the tobacco hornworm are almost entirely restricted to plants of the family Solanaceae. Oviposition appears to be initiated by olfactory stimuli widely distributed in this plant family, and consequently it is largely suppressed in the absence of host plants or after excision of the antennae. Both in the field and laboratory, the moths preferred tomato foliage over other solanaceous plants for oviposition. Larval feeding appears to be governed by gustatory stimuli common to plants of this family. On certain ones, such as *Nicandra* and *Petunia*, feeding and growth are limited by the presence of repellents or toxins. Some limited feeding occurred on certain nonsolanaceous plants after various periods of starvation.

The tobacco hornworm, *Protoparce sexta* (Johan.) is a host-specific insect of wide distribution throughout the tropical and temperate zones of the Americas. The larvae feed extensively on cultivated plants such as tobacco and tomato and to a lesser degree on members of the family Solanaceae (Rothschild and Jordan 1903, Moss 1920, Madden and Chamberlin 1945, Cary 1951). The adults suck on a wide variety of blossoms but molded perhaps by the regional abundance of certain species of plants, they oviposit selectively on a few out of the many plants which will support the larvae. Thus for example, in regions where tobacco is under extensive cultivation, this plant has been reported as the preferred host (Madden and Chamberlin 1945). Observations in the fields during two summers in Champaign county, Illinois, too revealed that only three plants, all under cultivation, were naturally infested. The plants were the tomato, *Lycopersicon esculentum* Mill., and two species of tobacco, *Nicotiana tabacum* L. and *N. affinis* L. Of these three plants, infestations of the hornworm were predominantly on tomato.

In studies of several host specific insects, other workers have reported that to a large extent chemical factors in plants governed the feeding behavior of the insects (Verschaffelt 1910; Dethier 1941; Chin 1950; Thorsteinson 1953, 1958; and others). The chemicals were demonstrated or implicated to act as feeding stimulants, feeding deterrents and toxins. Feeding stimulants are also known as attractants, token stimuli, and phagostimulants, and the distribution of any one feeding stimulant is generally limited to a family or to related families of plants. Correspondingly, an insect requiring a feeding stimulant is limited to plants containing the

[1]Portion of a dissertation submitted by the first-named author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology, University of Iilinois. This work was supported by a research grant from the Rockefeller Foundation. Accepted for publication December 11, 1959.

TABLE 1.—Oviposition record of tobacco hornworm moths when exposed to various solanaceous and nonsolanaceous plants.

Number of females	Plant		Percent distribution of eggs		Total eggs
			On plant	On cage	
8	*Physalis* sp.	(Solanaceae)	97.5	2.5	395
3	*Lycopersicon esculentum*	"	94.7	5.3	114
3	*Solanum dulcamara*	"	99.7	0.3	319
3	*Solanum tuberosum*	"	98.2	1.8	246
2	*Nicandra physalodes*	"	90.6	9.4	96
2	*Nicotiana tabacum*	"	94.5	5.5	166
2	*Capsicum annum*	"	90.6	9.4	64
2	*Lycium halimifolium*	"	90.7	9.3	43
2	*Datura Wrightii*	"	98.5	1.5	133
2	*Olea* sp.	(Oleaceae)	0	0	0
2	*Brassica oleracea*	(Cruciferae)	0	0	0
4	*Malus* sp.	(Rosaceae)	0	0	0
3	*Chenopodium album*	(Chenopodiaceae)	0	0	0
4	None		0	0	0

stimulant. In practice however, the presence of feeding deterrents and toxins has been shown to narrow further the actual available food plants (Chin 1950).

The study of the tobacco hornworm was initiated to determine whether chemical factors in its host plants also govern its feeding behavior and specificity. However preliminary experiments were first conducted to determine the extent of the ovipositional and feeding specificity of the hornworm on various plants. The feeding tests on the larvae were also useful in revealing plants which harbored repellent and toxic substances. The results of these experiments constitute the report given here.

MATERIALS AND METHODS

Hornworms used in the experiments were reared in the laboratory and were from stocks originally obtained from naturally infested tomato fields. Plants were gathered in the fields or were cultivated in greenhouses.

Ovipositional tests of the moths were conducted in a cheesecloth-covered cage which measured 3x6x3 feet. Experiments were of a single-choice or two-choice type: exposure to a single species of plant or to two species of plants. In either case, equivalent amounts of cut foliage placed 4 feet apart at opposite ends of the cage formed the choice stands. The moths were 3 days old and were used only once in the experiments. They were exposed to the plants overnight, or about 8 hours. Since oviposition occurs at dusk, a simulated dusk was created by focusing a table lamp with a 25-watt bulb onto the ceiling of the laboratory in which the cage was located. The eggs were removed from the foliage and counted the following morning.

TABLE 2.—Distribution of eggs of tobacco hornworm moths when the moths were provided with a choice between tomato and one other plant.

Number of females	Percent distribution of eggs					Total eggs
	On plant 1		On plant 2		On cage	
4	Tomato	50.5	Tomato	48.5	1.0	182
4	"	67.4	*S. dulcamara*	33.6	0.4	233
10	"	69.5	*D. Stramonium*	29.1	1.4	554
2	"	99.2	*N. physalodes*	0.8	0.0	121
3	"	76.5	*Physalis* sp.	21.1	2.4	213
4	"	71.5	*N. tabacum*	23.6	4.9	123
6	"	62.0	*S. tuberosum*	35.6	2.4	463
6	"	99.1	*C. annuum*	0.6	0.3	324
2	"	88.7	*Chenopodium album*	6.0	5.3	151
7	"	96.9	*Olea* sp.	3.1	0.0	262
3	"	89.5	*Malus* sp.	0.0	10.5	105
3	"	97.5	*Catalpa* sp.	0.0	2.5	199

The feeding performance of the larvae on various plants was tested by offering them 15 mm. discs stamped out from the leaves of plants. Five fourth- or fifth-instar larvae confined individually in small battery jars were used for each species of plant. The discs were examined after 1, 2, and 24 hours, and by comparing the amount of leaf discs eaten to that of control discs of tomato, the relative acceptability of each plant was determined. Preferences of newly hatched, unfed, first-instar larvae were determined by use of multiple-choice tests. For these tests, filter papers divided into eight equal parts were placed in 10-cm. diameter petri dishes. On directly opposite edges of each paper, 8-mm. discs of the same plant were placed so that four plants were tested at one time, each plant being represented by two stands. Ten larvae were then deposited in the center of each dish and allowed to wander and sample the leaf discs. Each test was replicated five times. The number of fecal pellets surrounding each disc after 24 hours was used as the criterion of acceptability of the plants.

RESULTS

Ovipositional Preferences of the Adults.—The requirements for oviposition are apparently met by most solanaceous plants since under single-choice situations, eggs were deposited only on these plants (table 1). However, under situations where the moths were able to express a choice between two plants, one of which was tomato, they showed a decided preference for tomato (table 2). These results indicate that at least two stimuli are operative in the manifestation of plant preferences by the adults. One stimulus, which is common to solanaceous plants, apparently triggers oviposition, while the other stimulus, peculiar only to tomato in this particular situation, may serve to orient the moths to the plant, but not to induce oviposition.

Presumably both stimuli operate olfactorily, although preliminary tests using crude ether extracts and steam distillates of the odorous components of tomato leaves were not fruitful in inducing oviposition. Antennectomy of the moths was also tried to find out whether by removal of the olfactory receptors, changes in the selection of plants might occur. The effect of antennectomy, however, was an almost total suppression of oviposition. Out of 21 antennectomized moths, a total of 79 eggs were obtained. These eggs were laid almost equally on the walls of the cage and on the host plants. The behavior of these moths suggested at least that they were not receiving stimuli necessary for oviposition and that these stimuli were probably of olfactory origin.

Feeding performance of the larvae.—Plants tested for their acceptability as food plants by the larvae are given in table 3. Whenever possible, these plants were also tested for their support of growth of the larvae. In general, these tests revealed that solanaceous plants as a group are readily acceptable in contrast to the nonacceptance of many randomly picked non-solanaceous plants, and that growth of the larvae on a plant appears to be dependent upon the initial acceptance of the plant. As an example, larval growth on solanaceous plants shown to be acceptable after 1, 2, and 24 hours of exposure

TABLE 3.—Feeding and growth performance of the larvae of the tobacco hornworm on various solanaceous and nonsolanaceous plants.

Plant	Acceptability[a]	Support of growth[a]
Group A. Solanaceous plants		
Lycopersicon esculentum	+++	+++
Datura Stramonium	+++	+++
" *innoxia*	+++	Not tested
" *ferox*	+++	Not tested
" *quercifolia*	+++	Not tested
" *meteloides*	+++	+++
" *arborea*	++	Not tested
" *Wrightii*	++	++
Lycium halimifolium	+++	+++
Nicotiana Tabacum	+++	+++
" *affinis*	+++	+++
" *rustica*	+++	+++
" *suaveolens*	+++	+++
Solanum nigrum	+++	+++
" *rostratum*	+++	+++
" *carolinense*	+++	+++
" *tuberosum*	+++	+++
" *tuberosum* (tuber)	++	+
" *Dulcamara*	+++	+++
" *melongena*	++	Not tested
" *Khasiana*	+++	Not tested
" *pseudo-capsicum*	++	Not tested
" *aviculare*	+++	Not tested
" *capsicastrum*	+++	Not tested
" *aculeatissimum*	+++	Not tested
Physalis virginiana	+++	+++
" *heterophylla*	+++	+++
" *ixocarpa*	++	Not tested
" *Alkekengi*	++	Not tested
Hyoscyamus niger	+++	Not tested
Atropa belladonna	+++	Not tested
Nicandra physaloides	+	+
Petunia hybrida	+++	Toxic
Capsicum annuum	++	++
Capsicum annuum (fruit)	++	+
Browallia americana	+++	Not tested
Brunfelsia americana	+++	Not tested
Acnistus arborescens	+++	+++
Group B. Nonsolanaceous plants		
Lactuca sativa L. (Compositae)	++	+
Brassica oleracea L. (Cruciferae)	++	—
Morus sp. (Moraceae)	+	—

[a] +++ = Acceptable within 1 hour of contact. Normal growth.
++ = Acceptable within 2 hours of contact. Slow growth and low mortality.
+ = Acceptable after 24 hours of contact. Slow growth and high mortality.
— = Unacceptable after 24 hours of contact. No growth.

TABLE 4.—Comparative growth of the larvae of the tobacco hornworm on various solanaceous plants after 12 days of feeding. Ten newly hatched larvae used initially.

Plant	Acceptability[a] of plant	Number of larvae surviving	Average weight per larva (gm.)
Solanum Dulcamara	+++	7	10.8
Solanum nigrum	+++	9	11.0
Nicotiana Tabacum	+++	6	11.2
Lycopersicon esculentum	+++	8	11.4
Datura Stramonium	+++	6	11.0
Datura Wrightii	++	8	5.7
Nicandra physaloides	+	3	2.4

[a]Acceptability of plants as recorded in table 3.

were tested for their support of growth. The results, given in table 4, show that on readily acceptable plants, growth of the larvae is equally good while on the less acceptable plants, growth is very prolonged.

The slight acceptance of some plants and the consequent poor growth of the larvae on them appeared to be due to feeding deterrents rather than to the poor nutritional quality of the plants. The possible presence of feeding deterrents in two slightly acceptable plants, *Nicandra physalodes* Gaertn. and *Datura wrightii* Hort. was hence tested by exposing larvae to these plants and by counting the number of fecal pellets deposited over 24 hours as an index of the feeding rate. The results, given in table 5, show that on acceptable plants, fecal pellets averaged 42 per fourth-instar larva while on *D. wrightii* the count was 18.9, and on *N. physalodes* 9.5. These results agree well with the results shown in table 4, in which growth of the larvae was dependent upon the acceptability of the plants initially. On the other hand, a highly acceptable plant, *Petunia hybrida* Vilm. was not able to support growth because of the presence of toxic substances. A larva ingesting *Petunia* foliage typically showed symptoms of vomiting, tremors, and prostration.

TABLE 5.—Fecal pellet count of third-instar larvae as a relative index of feeding on various solanaceous plants over a period of 24 hours.

Plant	Acceptability[a] of plant	Number of larvae	Fecal count per larva (average)
Lycopersicon esculentum	+++	11	41.6
Datura Stramonium	+++	10	43.0
Solanum nigrum	+++	10	42.6
Datura Wrightii	++	12	18.9
Nicandra physaloides	+	9	9.5

[a]Acceptability of plants as recorded in table 3.

Survival of intoxicated larvae was poor and death generally occurred in 24 to 35 hours.

Newly hatched first-instar larvae showed preferences for certain solanaceous plants which were not easily discerned in the older instars used in the acceptance tests. Thus under multiple-choice situations in which plants normally acceptable to older-instar larvae were used, tomato and *Solanum nigrum* L. were found to be the least preferred by the young larvae (table 6). Particularly in the case of tomato, it would appear that the adult ovipositional preference (table 2) for this plant in no way coincides with the feeding preference of the newly hatched larvae.

TABLE 6.—Relative acceptability of solanaceous plant by newly hatched first-instar larvae of the tobacco hornworm as measured by the distribution of the fecal pellets. The larvae were exposed to the plants under four-choice situations.

Total fecal pellets	Plant	Percent distribution of fecal pellets
927	*Lycopersicon esculentum*	5.8
	Physalis virginiana	52.1
	Nicotiana Tabacum	38.2
	Solanum nigrum	3.1
863	*Lycopersicon esculentum*	12.7
	Solanum Dulcamara	28.8
	Datura Stramonium	30.5
	Solanum tuberosum	27.9

Of the many nonsolanaceous plants tested for their acceptability to fourth- and fifth-instar larvae, only three were found to be acceptable to some extent. These plants were lettuce, cabbage, and mulberry (table 3). However, only lettuce was acceptable to newly hatched first-instar larvae. Lettuce was also found to be the only nonhost plant able to support growth, although growth was much prolonged and the larvae were very small at the prepupal stage. Prepupal larvae averaged 5.5 gm. compared to an average of 10.2 gm. for larvae reared on tomato or other acceptable host plants.

DISCUSSION

These preliminary experiments show that the manifestation of host specificity by the tobacco hornworm is assumed both by the adults and by the larvae. The adults appear to be more rigid in their specificity, since oviposition does not occur in the absence of host plants. The larvae, on the other hand, under stress of starvation, will not only attempt to eat foreign plants but will actually consume a few of them. Presumably, for oviposition and normal feeding, olfactory and gustatory stimuli common only to solanaceous plants appear to be involved.

The many species of solanaceous plants on

which the larvae can feed and grow normally indicate that the potential range of host plants is much greater than the number of plants which are infested naturally. This potential for infestation of a larger number of plants, however, appears to be largely circumscribed by the ovipositional preferences of the adults. In this case, the adults were shown to prefer tomato over other plants, but in tobacco-growing areas, tobacco has been reported as the preferred plant (Madden and Chamberlin 1945). Hence preferences appear to be regional and may be based upon the predominant available plant. The operative principle in host preferences of this type is commonly denoted as Hopkins Host Selection Principle (Hopkins 1917) and has been shown to be governed by olfactory conditioning of the preimaginal stages (Thorpe 1938; Thorpe and Jones 1937; discussed by Dethier 1954). The demonstration that the moths used in the experiments preferred tomato and the circumstance that the larvae were reared almost exclusively on tomato strongly indicates the operation of this principle in the tobacco hornworm.

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IDENTIFICATION OF THE NEWLY INTRODUCED PHLAEOTHRIPID HAPLOTHRIPS ?CLARISETIS PRIESNER (THYSANOPTERA)[1]

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ABSTRACT

The thrips tentatively identified as this African and Near-Eastern species may be either a useful pest of noxious plants or a detrimental crop pest, or both, in North America. It is distinguished from other thrips in its newly invaded territory principally by the relatively wide, medially constricted wings without accessory fringe hairs, and by the colorless, distally expanded major setae of head and pronotum.

An exotic species of *Haplothrips*, now tentatively identified as *clarisetis* Priesner, was found to have been in California since 1956 (Ewart and Brawner 1959) and in New Mexico since 1958 (Watts 1959). The discovery of this species and the size of its populations have aroused interest in its economic potential. Of the wide variety of plants on which it occurs in California, the largest populations are on Russian-thistle (*Salsola kali* L. var. *tenuifolia* Mey.); thus it may be useful in controlling this weed. In New Mexico it was causing commercial damage to lettuce. The following discussion is intended to permit the thrips to be recognized more easily, in order to help assess its capacity to damage either useful or noxious plants.

We have not been able to make certain that the newly introduced species is the same as the *clarisetis* of Africa and the Near East, but there is no doubt that it is exotic. No males have been found, and introduced species of thrips are usually parthenogenetic, because such forms are more easily introduced than those that reproduce sexually. More important, however, is the fact that the taxonomic affinities of the new species are with the native species of Africa, the Near East, central Asia, and Europe, and not with

[1]Accepted for publication January 6, 1960.

those of the New World or the western Pacific. The New World species closest to *clarisetis* are also doubtless introduced. Faure, who collected the two South African specimens in the type series and who has made a close study of *clarisetis* in South Africa (1955), confirms our opinion that the American specimens are probably *clarisetis*, but calls attention to certain differences between them and South African specimens (correspondence with W. H. Ewart, kindly made available to me). We do not know whether these differences fall within the range of variation in the South African population, or indicate a distinct species. If the introduced form is a geographical variant it may be possible to recognize where the introduction came from. Such a useful coincidence is unlikely, though, especially because the absence of males in the American population reduces the amount of morphological data available for comparison.

The series from which Priesner originally described *clarisetis* (1931) comprised two specimens each from Transvaal and Cairo, and there was no designation of holotype or type locality. Priesner seemed to consider the Transvaal specimens as "typical," but if there should be a recognizable difference between populations of the two localities, it is not fixed which should bear the name. In any case, the new American species is extremely similar to *clarisetis*.

Except where otherwise indicated, the following discussion applies equally to the American and South African forms. The illustrations are of the American form.

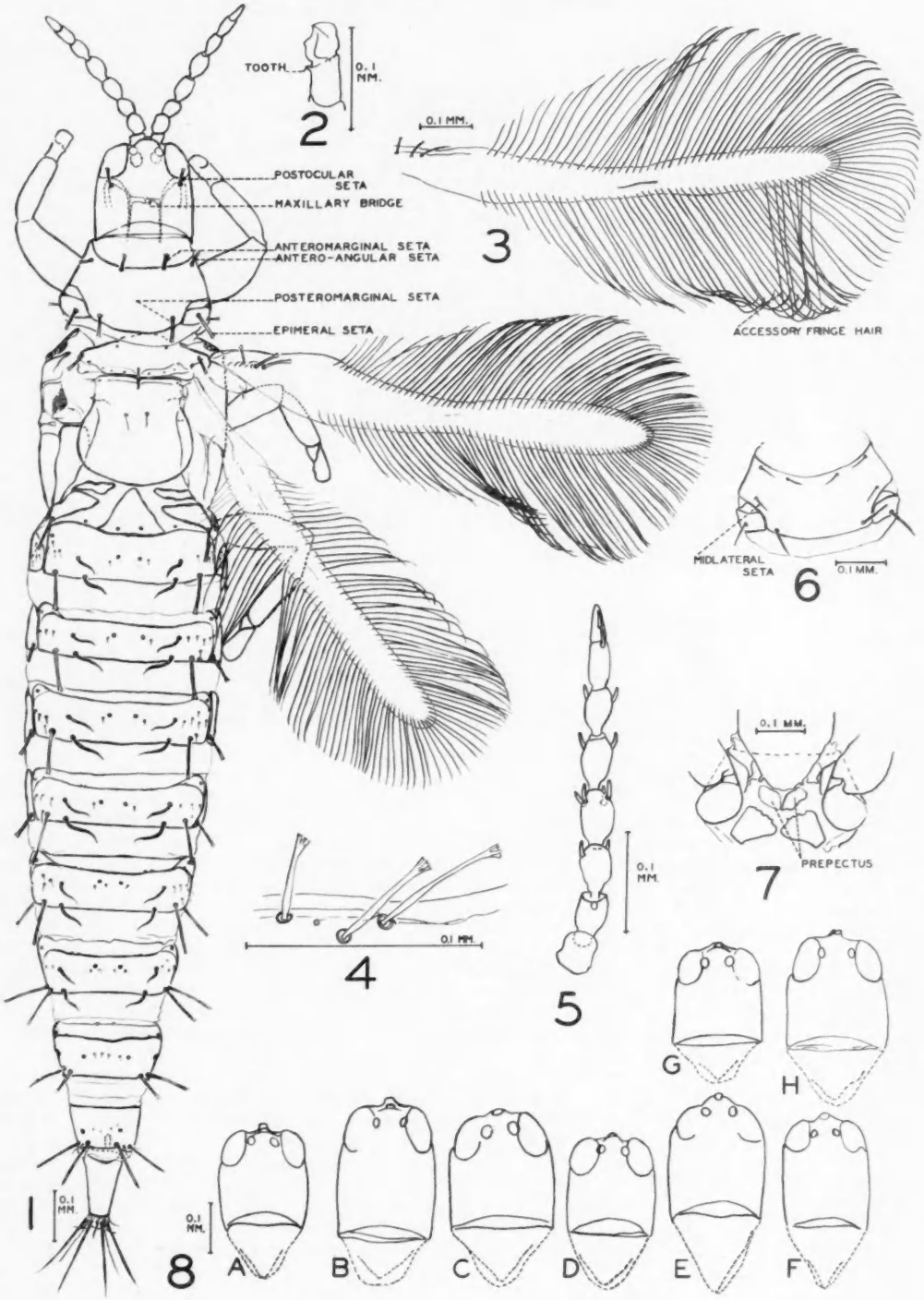
Haplothrips clarisetis is a reddish, dark-brown thrips, somewhat larger than the ordinary flower thrips, usually about 2 mm. long. The fore tarsus, the apex of the fore tibia, and the third antennal segment are brownish yellow; and the wings are colorless except for the brown fringe hairs and a small brown spot at the base of the fore wing. It can be distinguished from most North American thrips by the relatively wide, medially constricted wings, lacking accessory fringe hairs (compare figs. 1 and 3).

Probably the next most distinctive character of *clarisetis* is that which gives it its name—the stout, almost colorless setae of the head, pronotum, and wing base. The postoculars, anteroangulars and -marginals, epimerals, and posteromarginals (fig. 1), and the usual three wing-base setae (fig. 4) are well developed and widely expanded at the tip. Anteromarginals are present and midlaterals are absent, contrary to the most common situation in North American *Haplothrips*. An occasional specimen may have one or both setae of either pair reduced or well developed, a variation of the sort that makes the identification of a single specimen risky in any species.

No other thrips presently in North America combines the characteristics already mentioned, but there are many additional characteristics of *clarisetis* that may be observed for a more certain identification. The medially constricted wings, the slightly oblong-rectangular head (figs. 1 and 8), the maxillary bridge (fig. 1), the rounded mouth cone (fig. 8d), and the prepectus (fig. 7) are characteristic of the genus *Haplothrips*. Some of the native species that most resemble *clarisetis*, those of the *fasciculatus* (Crawf.) complex, may have the fringe hairs at the tip of the wing white, contrasting with the brown fringe hairs of the remainder of the wing; the metanotum longitudinally striate, instead of faintly reticulate as in *clarisetis*; or the internal pigment purple, rather than red as in *clarisetis*. Few native species of *Haplothrips* have more than one sense cone on the third antennal segment; *clarisetis* has two (fig. 5), as do most of the introduced species. The tarsal tooth much used in the identification of *Haplothrips* species is so small and so easily concealed in females of *clarisetis* (fig. 2) that its presence is of no use in their identification, except as opposed to species with a particularly prominent tooth. South African males have a distinct tooth. The head and mouth cone differ somewhat in shape from those of other common *Haplothrips* (fig. 8) (these species, except for *graminis* Hood, occur in the western part of the country), but probably the only useful differences are the pointed mouth cone in *distalis* Hood and *malifloris* Hood, and the long head in *cottei* (Vuillet). The discal pores and minor setae of abdominal terga VII and VIII of *clarisetis* are most often as shown in figure 1, but the setae vary in number from 2 to 5 and occasionally one or both of the pores are lacking.

EXPLANATION OF FIGURES

1. *Haplothrips ?clarisetis*, dorsal aspect. Left pterothoracic appendages omitted, also most of minor setae and other detail.
2. *H. ?clarisetis*, fore tarsus, showing small tooth on inner side.
3. *H. halophilus* Hood, right fore wing, showing usual width of *Haplothrips* wing, and accessory fringe hairs.
4. *H. ?clarisetis*, basal wing setae, characteristically dilated and frayed at tip.
5. *H. ?clarisetis*, dorsal aspect of antenna; minor setae omitted.
6. *H. malifloris* Hood, pronotum; midlateral setae well developed.
7. *H. ?clarisetis*, prosternum, showing prepectal plates.
8. Outline of head and mouth cone in dorsal view. A. *H. faurei* Hood. B. *H. cottei* (Vuillet). C. *H. fasciculatus* (Crawf.). D. *H. ?clarisetis* Pr. E. *H. malifloris* Hood. F. *H. distalis* Hood. G. *H. graminis* Hood. H. *H. halophilus* Hood.


TOOTH
0.1 MM.
2
0.1 MM.
3
ACCESSORY FRINGE HAIR
POSTOCULAR SETA
MAXILLARY BRIDGE
ANTEROMARGINAL SETA
ANTERO-ANGULAR SETA
POSTEROMARGINAL SETA
EPIMERAL SETA
MIDLATERAL SETA
0.1 MM.
6
0.1 MM.
PREPECTUS
7
0.1 MM.
4
0.1 MM.
5
0.1 MM.
1
0.1 MM.
8
A
B
C
D
E
F
G
H

A species that might receive especial mention is *cottei* (Vuillet), the carnation pest of Mediterranean countries, which has been introduced into Mexico and the Brownsville area of Texas. This thrips is unique in that the wings may vary in any degree from short to long and, in the fully macropterous form, may have from one to eight accessory fringe hairs or none at all. Unless this anomaly is recognized, it causes confusion. The fully macropterous form lacking accessory fringe hairs resembles *clarisetis* closely; in fact, *cottei* belongs to the same or a closely related species complex. In any of its forms *cottei* is readily distinguished from *clarisetis* by the major setae, usually having only the postocular, posteromarginal, epimeral, and wing-base setae well developed, and all of these pointed or nearly so. Unlike almost all other introduced *Haplothrips* in North America, *cottei* produces males.

Males of *clarisetis* have not been found in North America, and are not expected to occur. If males should be discovered that have other characteristics of *clarisetis*, a comparison of the genitalia should show definitely whether or not the American form is identical with the South African. An excellent description and figures were given by Faure (1955).

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EVIDENCE FOR A POTENT SEX ATTRACTANT IN THE INTRODUCED PINE SAWFLY, DIPRION SIMILIS (HYMENOPTERA: DIPRIONIDAE)[1]

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ABSTRACT

In field investigations virgin females were found capable of attracting exceptionally large numbers of males. One caged female attracted well over 11,000 males. The chemical properties of the attractant are under investigation. Such an attractant in a stable form, and in combination with insecticides, might aid in reducing populations of this arrhenotokous pest.

Chemical stimuli, in the form of odors or scents, play an important role in the lives of most insects. The stimuli which act as attractants may be divided generally into those concerned with sex and those concerned with food. With the development of insecticide resistance by some insect species, the concern over the harmful effects of insecticide residues, and the hazard to wildlife in general insecticidal applications to forests, the possibility of utilizing sex attractants in insect control has received considerable impetus. Recent reviews of insect repellents and attractants by Hall et al. (1957) and on pheromones in insects by Karlson and Butenandt (1959) have indicated the complex phenomena involved. To date, most of the research on sex attractants has been done in the order Lepidoptera, but some has also been initiated with insects of the following orders: Hemiptera (suborder Heteroptera), Orthoptera, Coleopter, and Hymenoptera (suborder Clistogastra) (Karlson and Butenandt 1959). Apparently no investigations have been undertaken on the extensive group of hymenopterous defoliators in the suborder Chalastogastra. The introduced pine sawfly *Diprion similis* (Htg.), an arrhenotokous sawfly pest of eastern white pine, *Pinus strobus* L., in the United States, belongs to the latter group and is the subject of this report.

During field studies on the biological control of *D. similis* in Wisconsin considerable data on the mating habits were accumulated. The only published reference to the act of mating in this species was that of Britton and Zappe (1918) who observed copulation once. Apparently, however, mating has been observed under laboratory conditions on numerous occasions by investigators both in Canada and the United States. Mating was observed frequently in nature during the present study and was evidenced by the assembling of large numbers of males which swarmed

[1]Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This work was done with the support of the Wisconsin Conservation Department and presented at the joint meeting of the Entomological Society of Ontario, Entomological Society of Canada and the Entomological Society of America held at Detroit, Michigan, Nov. 30–Dec. 3, 1959. Accepted for publication January 6, 1960.

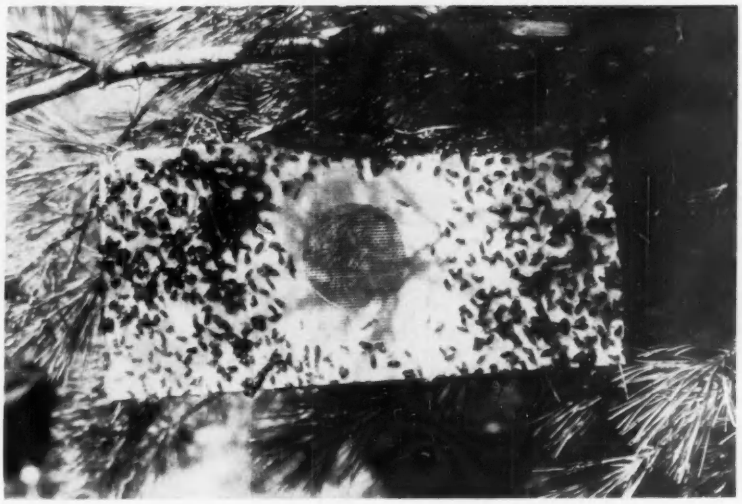

FIG. 1.—Wooden trap with virgin female of *Diprion* showing attractiveness to males.

toward the female from the surrounding area. Females emerging from ground-level cocoons were usually mated by the time they had crawled part way up the stems of grasses. Those emerging from cocoons spun on the tree branches were usually mated in the vicinity of the empty cocoon. Mating was always observed after the meconium had been discharged. Once a female sawfly was mated she no longer elicited a response from the males.

Because of the initial response of such large numbers of males to newly emerged females, investigations were conducted to determine whether these biological relationships might be useful in attempting a male eradication program such as has been used for certain other insect species. A wooden trap (fig. 1) designed to prevent mating of the females was utilized. It consisted of a board, 12x6x1 inches, with a 2½-inch screened hole in the centre. The female was placed in the screened portion and an adhesive (tree tanglefoot, The Tanglefoot Co., Grand Rapids, Mich., U.S.A.) was spread over the wooden portion. These traps were suspended from trees in infested areas.

In the first series of experiments the traps were baited with one virgin female each and controls were set up, some as empty traps and some with virgin males. The control traps attracted no significant numbers of males, though seven were found on one trap. The virgin female traps, on the other hand, attracted an average of 1,000 males each (data from eight traps) with a range from 542 to 1,706. These numbers are the actual counts made and do not take into consideration the large numbers of males which fell to the ground. Some variation in the attractiveness of the females to the males exists, as several females did not attract males. The reason for this is not known. The response by the males to virgin females was rapid and many approached within 30 seconds after the cage was set up. Wind appeared to be an important factor in attracting large numbers of males, as those traps set at an angle of 90° to the wind direction at the edge of the woods were consistently more attractive than those set in the dense portion of the woods. No significantly different male catches were recorded when virgin females were set up according to the cardinal points of the compass about a single tree. The greatest activity took place from mid-morning to mid-afternoon, and no male movement was observed in the early evening hours before sunset even though high temperatures prevailed. One record of attractiveness not included in the data above is particularly outstanding. A virgin female was set out at 11 a.m. C.D.T. and the first males flew into the trap within 30 seconds. Activity continued until 4 p.m. at which time over 7,000 males had been attracted. This female continued attracting males at approximately 1,000 per day until she died on the fifth day. Even after death there was sufficient attractant left to lure males in dwindling numbers for the next 3 days. In all, over 11,000 males were removed from the trap, and the ground below was littered with many not included in the count. Though it is not known from what distance males are lured, it was possible to attract them approximately 200 feet out of the forest over an open field. Because of high populations in the infested area it is unlikely

that flights of this length are normally undertaken.

Realizing the potency of the attractant produced, an attempt was made to locate the position of the glands producing this chemical. Heads and thoraces, and abdomens of virgin females were crushed separately between pieces of filter paper which were then placed in the traps. In the first series, traps were placed within 12 inches of one another and though the trap containing the crushed abdomens attracted twice as many males (3,238:1,681) as did that with the heads and thoraces, the data are not too reliable because of the flight pattern of the males. In the second series, the traps were placed approximately 10 feet apart. The crushed abdomens attracted 270 males and the crushed heads and thoraces only 25. Interestingly enough, the traps containing crushed abdomens remained attractive for 25 days, the attractiveness dwindling during the last week. Mashed abdomens from virgin females 6 days old were not as attractive as those from newly emerged females. It was concluded that the attractant was produced by abdominal glands and that any attraction by the heads and thoraces resulted from contamination.

The third series of experiments was set up to determine whether the chemical involved could be extracted. An attempt to trap the attractant by passing air rapidly over virgin females and then through various solvents was unsuccessful. Crushed, whole, virgin females and control males were extracted in numerous organic solvents. Field assays utilizing two female or two male equivalents in each solvent and an additional control trap (empty) were undertaken. Only the extracts from two female equivalents in benzene or acetone gave indications of attractiveness to males, the latter capturing the greater number (61:16). The benzene fraction did not attract males until the third or fourth day whereas the acetone fraction attracted weakly the first day and strongly for the next 5 or 6 days. It is not known whether the filter paper had any binding effect on the extracts. During these tests it was observed that if newly-emerged virgin females were left in plastic dishes with tight fitting lids until all were dead and then the dishes were opened in the field, many males were attracted. The males attempted copulation with the dead females as well as with males that had been contaminated within the dish. It was also possible to attract males to these dishes after the females had been removed. This suggested that a deposit, attractive to males, was present in the container.

The next series of experiments attempted to answer the question of whether it would be possible to extract the sex attractant by either surface-scrubbing the virgin females after death or by rinsing the containers in which they were held. Large numbers of fresh virgin females were confined 50 to each sterilized glass container and left until all the females were dead. Numerous solvents were used to rinse the glass containers and surface-scrub the adults to obtain an attractive extract. Field assays were then made in the infested area. The sex attractant in the ether fraction was found to elicit the greatest response from the males. Though the benzene fraction elicited some response from the males it was not nearly as marked as that from the ether fraction. The chemical nature of this attractant is now under investigation, but the development of a satisfactory laboratory assay method has yet to be solved. Caged males do very little flying and it is not known whether this is important in developing a quantitative assay method. Preliminary observations with caged males and virgin female sawflies, under both laboratory and field conditions, indicate that, when flying does not occur, considerable circling takes place prior to mating. This may provide a basis for laboratory assay.

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ANNUAL MEETING ENTOMOLOGICAL SOCIETY OF AMERICA

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SUPPRESSION OF GRASSHOPPERS BY NEMESTRINID PARASITES (DIPTERA)[1]

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ABSTRACT

This paper supplements an earlier (1952) report on parasitization of *Metator pardalinus* (Sauss.) by *Trichopsidea clausa* (O.S.) in Montana, but deals chiefly with the effect of *Neorhynchocephalus sackenii* on a host population of *Melanoplus bilituratus* (Walk.) in eastern Oregon in 1955–56. On a 200,000-acre area, a 99% reduction of an outbreak of *bilituratus* coincided with heavy parasitization by *N. sackenii*, when 70% of the hosts taken in net collections were found parasitized. Decline of the host population resulted from shortened life span and reduced egg production by parasitized individuals. In cage tests, parasitized *M. bilituratus*, *M. bivittatus* (Say), and *M. femur-rubrum* (DeG.) lived not more than half as long as unparasitized individuals, and their egg production was reduced by 93%, 49%, and 95%, respectively. Though *M. bivittatus* was found heavily parasitized in the field, apparently it is not a true host of *N. sackenii* as none of the fly larvae reached maturity in 180 specimens kept under observation. Parasitization of *M. femur-rubrum* was accomplished under laboratory conditions, by placing a *sackenii* larva on the abdomen of each host and watching it until penetration was complete.

There is little published information on the effective potential of nemestrinid parasites in the suppression of grasshopper populations. This paper is chiefly a report of a field study of parasitization in 1955 and 1956 on Oregon rangelands by *Neorhynchocephalus sackenii* (Will.), but includes a discussion of parasitization by *Trichopsidea clausa* (O.S.) based on earlier studies conducted in Montana. These are the only two nemestrinid species in the United States or Canada known to parasitize grasshoppers. A study of the comparative mortality and fecundity of parasitized and unparasitized hosts kept in cages is also included.

REVIEW OF THE LITERATURE

The first report of parasitization of grasshoppers by a nemestrinid was by Potgieter (1929), who in 1925 found larvae of *Trichopsidea* (*Symmictus*) *costata* (Loew) parasitizing the brown locust *Locustana pardalina* (Walk.) in Bechuanaland, South Africa. Potgieter commented as follows: "Often female locusts were found with the larva present and yet containing almost mature eggs. It appears as if the parasite is not at all deadly to the host, and although it may shorten its life, it does not prevent the locust from oviposition. . . . From the information we have concerning this fly, it would appear that, under favorable conditions, it takes at least about a year to complete its life cycle and this lessens its efficiency as a locust parasite."

Spencer (1931, 1932), in the Riske Creek area of British Columbia, noted light-colored flies ovipositing in crevices of poles of a telephone line crossing egg beds of the grasshopper *Camnula pellucida* Scud. The flies were identified by Bequaert (1934) as *Trichopsidea* (*Parasymmictus*) *clausa* (O.S.). He cited Spencer's observations as the northernmost record of any species of Nemestrinidae. Spencer (1945) reared this fly from larvae emerged from *pellucida* and he and E. R. Buckell reared *N. sackenii* from larvae emerged from *Melanoplus mexicanus* (Sauss.), now known as *bilituratus* (Walk.), taken near Kamloops on British Columbia cattle ranges.

Long before any of these observations were made, Olliff (1891) recorded the finding in New South Wales of a large dipterous larva in the plague grasshopper *Chortoicetes terminifera* (Walk.). Olliff included a drawing of the mature larva in his report, but he did not rear out the fly, and it was not determined to be a nemestrinid until Noble (1936) observed that maggots obtained in 1935 from plague grasshoppers in New South Wales were "apparently similar to that figured by Olliff in 1891." Noble succeeded in rearing flies from the parasite maggots and identified them as *Trichopsidea oestracea* Westwood. He also found this nemestrinid parasitizing the wingless grasshopper *Perelytrana rana* Sjöst. His report made no mention of the percentage of parasitization in host populations.

Fuller (1938) made a more detailed report on the same nemestrinid parasite in the same Australian territory. Mature nemestrinid larvae were discovered in grasshopper cages when soil kept in them was sifted to recover grasshopper egg pods. Fuller listed four additional species from which the parasites were recovered, *Phaulacridium vittatum* (Sjöst.), *Gastrimargus musicus* (Fabricius), *Austroicetes pusilla* (Walk.), and *Macrotona securiformis* (Sjöst.). Average parasitization of the four species was estimated at 5 percent.

Crouzel and Salavin (1943) found grasshoppers of the species *Dichroplus arrogens* (Stal) and

[1]Accepted for publication January 12, 1960.

[2]The author is grateful to C. P. Clausen, formerly of the Citrus Experiment Station at Riverside, Calif., and F. T. Cowan, of the U.S.D.A. Grasshopper Research Laboratory at Bozeman, Mont., for suggestions in preparation of this manuscript.

elongatus G. Tos in Argentina parasitized by larvae of two closely related nemestrinids, *Neorhynchocephalus sulphureus* (Wied.) and *vitripennis* (Wied.). Parasitization ranged from 5 to 16 percent. Their paper describes some of the life stages of *sulphureus* but gives few data on its habits and life history. Concerning the effect of parasitism upon the host's longevity and reproductive capacity they made the following comment: "From histological dissections we have confirmed that the larva is not essentially haemophagous but is nourished from the host's fatty tissues, even in its early stages; never have we found damage either to vital organs or to eggs in the host. We have not seen, as cited by Potgieter, the oviposition by a parasitized female. It could be that this [effect upon host oviposition] depends upon the relative size of the host to the parasite."

Greathead (1958) reported on parasitization in Ethiopia and Kenya, Africa, of the large desert locust *Schistocerca gregaria* (Forskål) by the nemestrinid *Symmictus* (*Trichopsidea*) *flavopilosa* Bigot. He found that the parasite destroys the host's fat-body, impairs its ability to ingest food, and finally kills the host upon emergence. Parasitization takes place only in the host's nymphal stages, and the full-grown larvae usually leave the hosts in the latter's fourth instar. Maximum parasitization was 34 percent.

OBSERVATIONS IN MONTANA AND ARIZONA

In some locations outbreak grasshopper populations have been noted to undergo drastic reduction after severe attack by nemestrinid parasites. York and Prescott (1952) reported heavy parasitization of *Metator pardalinus* (Sauss.) by larvae of *Trichopsidea clausa* on a cattle range in southeastern Montana in 1949 and 1950. Since the effects on the host population were only partially covered in that report, they are herewith summarized as follows:

Counts made on July 13, 1949, showed an infestation of *paradalinus* on a square mile of western wheatgrass range to number 5 to 12 adults per square yard. Thirty-one percent of the females were found to be parasitized by *clausa* larvae on that date. At termination of the host's oviposition period, sifting of soil revealed an average of 1 egg pod per square foot.

This heavy oviposition resulted in a severe outbreak of *pardalinus* in the following year. Counts made on June 20, 1950, when *pardalinus* was in the third and fourth instars, ranged from 50 to 75 per square yard. On this date many *clausa* flies were found ovipositing in beetle holes and weather checks of juniper fenceposts and other wooden structures in the area. They were congregating on posts over most of two lines of fence, including a quarter-mile stretch on the south margin as well as a shorter remnant of about 30 posts near an old windmill where cattle congregated to water. Counts of ovipositing flies made on June 21 showed 44 to 107 per post. By mid-July *pardalinus*, which was then adult, numbered 8 to 10 per square yard. On August 12 net collections were examined for nemestrinid parasites, and *clausa* larvae sufficiently large to be seen without magnifying aids were found in 80 percent of the females. After the oviposition period an hour of soil sifting by two observers yielded but a single egg pod, indicating the probability of a greatly reduced host population in the ensuing year.

When the area was visited again on June 9, 1951, *paradalinus*, then chiefly in the fourth instar, averaged one per 4 square yards, indicating a population reduction from the previous year in excess of 99 percent. The *clausa* flies from the overwintered larvae were beginning to emerge in large numbers. Counts of ovipositing females averaged 10 per post. It was early in the fly-emergence period and probably their numbers increased considerably during the next 2 weeks. Owing to scarcity of hosts, observations at this location were not continued beyond this date.

Extreme reductions of *paradalinus* populations following intensive parasitization by *clausa* maggots were reported by York (1955) for other southeastern Montana locations. Fairly high parasitization by *clausa* in the grasshoppers *Arphia pseudonietana* (Thomas) and *Trachyrhachis kiowa* (Thomas) was noted in 1949 in southeastern Montana, but its effect upon the host population was not studied (York and Prescott 1952). No evidence of parasitization by *clausa* in *Melanoplus* species has ever been reported.

Neorhynchocephalus sackenii is similar to *clausa* in general appearance and habits. The larvae have the same destructive effect upon the host's reproductive capacity. The flies of the two species, as seen in the field, appear of like size and coloration, and their movements, buzzing sounds, and general behavior are practically identical. On close inspection, however, the *sackenii* fly is distinguishable by its long stiletto-like proboscis. In *clausa* this is short, truncate, and concealed beneath a dense pile. The life cycles of the two species are similar, and the full-grown larvae of both are characterized by their large size as compared with their hosts (fig. 1).

N. sackenii has been found parasitizing a fairly wide range of grasshopper species belonging to several genera. Included are some of the more important *Melanoplus* species, *bilituratus* being a major host. Both the number of its hosts and the wide distribution of some of them probably account for the fact that *sackenii* is found more generally throughout the country than is *clausa*. In the Midwestern States it is found not only on the semi-arid rangelands occupied by *clausa*, but is equally abundant in the highly humid coastal

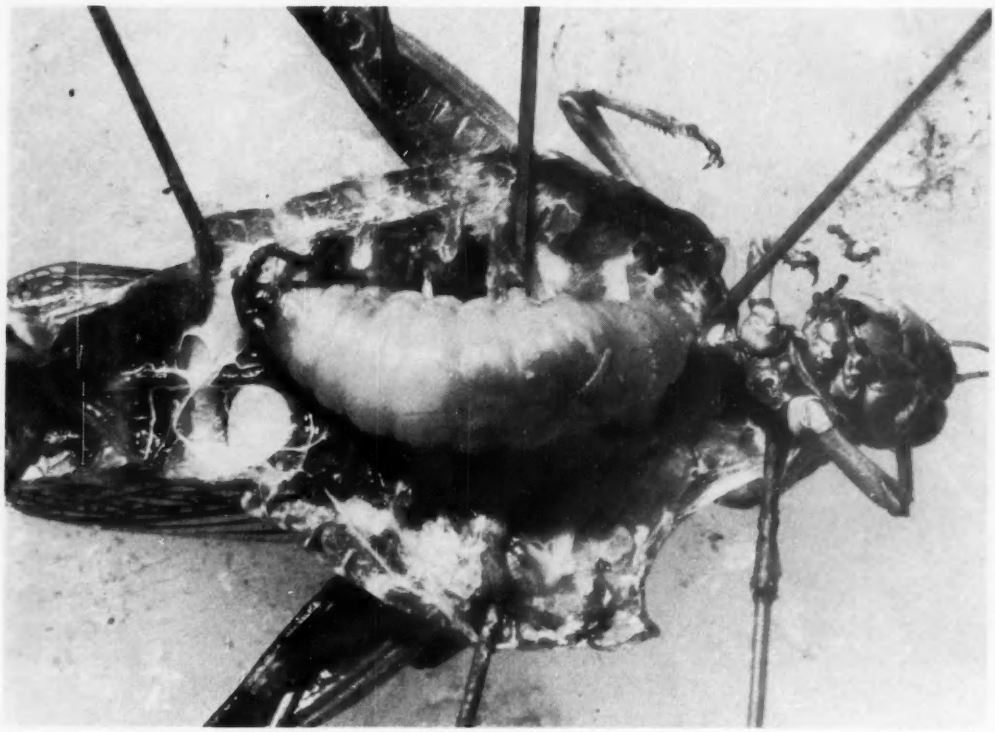

FIG. 1.—Dissected *Melanoplus bilituratus* female with mature *Neorhynchocephalus sackenii* larva in situ.

region of the Pacific Northwest. In the Northern States it ranges from sea level to elevations of 5,000 feet.

Light to moderate parasitization of several grasshopper species in Montana localities has been reported (York and Prescott 1952, Prescott 1955). Heavy parasitization was found by York (1955) in *Ageneotettix deorum* (Scudd.), 76 percent of which contained *sackenii* maggots.

More recently Nerney (1957) made studies of parasitization by *sackenii* of two grasshopper species that are of economic importance on Arizona rangelands, *Boopedon nubilum* (Say) and *Morseiella flaviventris* (Bruner). In 1957 he found parasitization of these hosts running as high as 57 percent. This is the first record of nemestrinid parasitization of these species.

Neorhynchocephalus sackenii A MAJOR FACTOR IN DECLINE OF *Melanoplus bilituratus*

In 1955 and 1956 heavy parasitization by *sackenii* in a severe and extensive outbreak of *bilituratus* was observed on rangelands in Morrow County, 40 miles southwest of Pendleton in north-central Oregon. The county's northern boundary is the Columbia River. From low benchlands just above the river level the terrain rises from 240 feet to elevations exceeding 5,000 feet, where the Blue Mountains intrude into the southern end of the county.

As a sort of vanguard to the forested Blue Mountain summits is a broad cordon of steep, treeless hills extending from east to west across the central portion of the county. The summit areas of these hills range from 2,000 to 4,000 feet and include rangeland that in some years gives rise to heavy grasshopper populations. The annual precipitation along the lower benchlands flanking the Columbia River averages 10 inches, and increases to 20 to 25 inches on the higher rangeland. There is a corresponding summer and fall precipitation gradient (June through November), from 4 inches on the benchlands to 9 inches on the rangeland summits. At elevations below 2,000 feet conditions usually become too dry in late summer and fall to support large grasshopper populations, but above this level they approach optimum for grasshopper propagation.

The principal *bilituratus* infestation was in eastern Morrow County, extending 30 miles eastward from Heppner to Vinson in Umatilla County. In the USDA forecast the 1955 grasshopper infestation for this area of about 200,000 acres was

FIG. 2.—Typical rangeland vegetation in area of heavy grasshopper infestation, Morrow County, Oregon.

designated as "very severe" (14 to 28 grasshoppers per square yard). The elevation is 1955 feet at Heppner and rises to 3,848 feet near the central portion of the area. The vegetation is composed of mixed range grasses and forbs. Rabbit brush (*Chrysothamnus* sp.) is generally prevalent, but other shrubs and trees are scarce (figs. 2 and 3).

When the area was first visited, on July 12, 1955, a stop was made 10 miles east of Heppner where *sackenii* flies were seen emerging in large numbers. *M. bilituratus* was generally abundant and mostly in the fourth and fifth instars. When this area was examined 6 weeks later, *bilituratus* was in the adult stage. The grasshoppers, seeking the less parched segments of the range for feeding and egg laying, were concentrated chiefly on the north slopes and in the numerous descending troughs of the convoluted hills (fig. 3). In these troughs and slopes, which comprised an almost unbroken series for a distance of 30 miles, populations averaging 8 to 12 per square yard were heavily parasitized by *sackenii* larvae.

Net collections of *bilituratus* were taken on August 24 at irregular intervals for 29 miles across the range. The parasitized grasshoppers in such collections, determined by examination for *sackenii* respiratory vent marks in the area of the host's abdominal spiracles, ranged from 35 to 98 percent and averaged 70 percent for the entire population. In a previous account (1955) the author erroneously concluded that these vent marks were caused in part by larval penetration of the host. It was subsequently determined, however, that the larva leaves no mark at the point of entry into the host.

In addition to *bilituratus*, light populations of *bivittatus* were present in some of the major troughs. *N. sackenii* parasitization was very heavy in this grasshopper wherever it was encountered, and in sexually mature adults was close to 100 percent. The few unparasitized adults that were collected were in a callow condition. Since most parasitization takes place after the host has attained the adult stage, it seemed only a matter of time that the few hosts that had so far escaped must become parasitized also. Of 43 *bivittatus* specimens collected 11.3 miles east of Heppner, 42 were parasitized. In a 7-mile trough between Vinson and Butter Creek summit the grasshopper population was almost exclusively *bivittatus*. At the upper end of this trough, 35 of 37 of this species collected were parasitized. A few miles farther down the trough, 18 of 26 were parasitized, and six of the eight unparasitized hosts were in a callow condition. A third collection taken another mile farther down had 8 out of 10 parasitized.

The severity of *sackenii* attack upon *bivittatus* was indicated not only by the high percentage parasitized but also by the number of larvae pene-

FIG. 3.—Typical rangeland terrain with alfalfa bottom, Morrow County, Oregon.

trating individual hosts, some of which had as many as nine respiratory vent marks. The 36 parasitized *bivittatus* taken at the upper end of the 7-mile trough had an average of four marks each. Similar though less extreme evidence of multiple penetration was found in *bilituratus*.

A total of 180 parasitized *bivittatus* grasshoppers were collected and kept outdoors in a screen cage until late fall. By this time most of them had succumbed. Although they were fed regularly and otherwise well cared for, not a single *sackenii* larve emerged from these grasshoppers. Numerous dead hosts were dissected and found to contain the remains of badly decomposed *sackenii* larvae. Many such larvae had nearly attained mature size before succumbing. From this and subsequent tests it became evident that *bivittatus* is not a true host of *sackenii*, since it does not perpetuate the parasite.

The Morrow County area was observed again on July 6, 1956. At this time emergence of sackenii flies was well under way. The host population throughout the area showed at least 95 percent reduction from the previous year. *M. bilituratus* nymphs averaged only one per 4 square yards, and they were in the third to fifth instars. When the area was visited again a week later, a few individuals had reached the adult stage and *sackenii* flies were two to three times more abundant than during the previous visit.

On one location 50 flies were collected while they were taking nectar from the heads of yarrow, *Achillea millefolium* (white blooms in foreground of fig. 2), a fairly prevalent plant throughout the area. The flies were ovipositing in nonconformity to the usual behavior reported for nemestrinid species, avoiding the numerous beetle-riddled and weather-checked fenceposts and telephone poles transecting the terrain. They showed a distinct preference for cavities and crevices in dead or moribund portions of the range vegetation. They were found ovipositing in the vacated cavities of several kinds of small trypetid galls found on rabbit brush and in larger polythalmous galls on wild rose (fig. 4). They were much attracted to clumps of giant wild rye grass (*Elymus condensatus*), ovipositing in the spikes and in end cavities and under leaf sheaths of old cropped or broken culms. Their egg clusters were found in the hollows of old dead stems of wild salsify (*Tragopogon porrifolius*), in hollow, cropped-off stems of *Eriogonum heracleoides*, in end cavities of broken dead stems of thistle (*Carduus* sp.) and St. Johnswort (*Hypericum perforatum*) from which the pith had been removed by wasps, and in old hollow seed pods of dead plants of both mullein (*Verbascum thapsus*) and St. Johnswort.

The heavy fly emergence and oviposition was followed by an intensive larval attack upon the already greatly reduced grasshopper population.

However, as would be expected, parasitization was somewhat lower than in the previous year. A collection of 134 *bilituratus* taken at one location on August 3, when the host was in the adult stage, was 58 percent parasitized. The hosts were so few that they had to be sought, pursued, and captured individually. By August 20, as determined by cage tests, matured larvae began to emerge from their hosts, killing them in the process, and emergence continued at peak rate until about September 10. This caused an abrupt midseason decline in the host population. Even by September 6 the *bilituratus* population had fallen to one in 20 square yards from one in 5 square yards a month before.

This midseason decline in hosts was evident throughout the area. An effort was made to determine the percentage of parasitization in all the locations checked the previous year, but grasshoppers were so scarce that in some of the troughs only an occasional individual could be seen. On the basis of the few individuals that it was possible to capture, parasitization was found to average 58 percent in *bilituratus* and 71 percent in *bivittatus*.

Surveys of Morrow County rangeland for determination of grasshopper populations and egg deposition were made in 1954 and 1955 by the Plant Pest Control Division. Figure 5, based upon data taken from their field reports, shows an average deposition in mid-October 1954 of 0.6 pod per square foot by a grasshopper population of 13 per square yard. This heavy egg deposition resulted in a dense population in 1955, which their August adult survey indicated as 29 per square yard. This was the population that the author found to be heavily parasitized by *sackenii* maggots. Despite the fact that the 1955 grasshopper population, according to the survey data, was more than twice as heavy as the 1954 population, its egg deposition was only one-sixth as heavy. This fact explains the low grasshopper population indicated for 1956, with an egg deposition too low for determination.

N. sackenii reached its peak of abundance, as determined at the time of fly emergence, not during the 1955 peak of host abundance, but in 1956 after the host population was sharply reduced. Although a maximum number of mature *sackenii* larvae were produced during the year of maximum host abundance, they overwintered in the soil and did not emerge as flies until the following summer. The consequent heavy *sackenii* attack on the already depleted host contributed toward its further decline to near-zero level in 1957.

Nemestrinid parasitization is, in general, a continuing process in which the free larvae are seeking and finding their hosts over a period of several weeks. For this reason the proportion of the host population that ultimately became parasitized must have been much larger than the percentages herein indicated, which are based on samples of the surviving population taken on given dates.

NEMESTRINID PARASITIZATION A RANGELAND PHENOMENON

Much remains to be learned concerning the factors that favor the propagation of nemestrinid parasites. It is certain that more than the mere presence of a host population is essential. The author has encountered nemestrinid parasitization of grasshoppers on certain types of rangeland habitats or on idle lands that have been out of cultivation for years, but never on croplands or cultivated areas in general. A typical area is illustrated in figure 3. The dark, flat area crossing the picture at the base at the hill slope is an old, run-down alfalfa field. In 1955 this field was almost surrounded by native rangeland supporting a *bilituratus* population that was 32 percent parasitized by *sackenii* larvae; yet none of the *bilituratus* taken from the alfalfa was parasitized. The same condition was found in other fields of the area, including those of both grain and alfalfa. These observations indicate not only that the ovipositing *sackenii* flies avoided the cultivated crop lands, but also that there was negligible migration of the parasitized hosts to these lands from the surrounding range.

EGG PRODUCTION AND MORTALITY OF PARASITIZED GRASSHOPPERS

Early in August 1956 three grasshopper species parasitized by *sackenii* maggots were placed in screen cages, and records of their egg production and mortality were obtained until the end of November. Cages of unparasitized grasshoppers of the same species were used as controls. All grasshoppers used were young adults which had not yet begun oviposition. Males as well as females were included in each cage to normalize conditions as nearly as possible.

The cages were made of ordinary window screen covering four sides and half the top of wooden frames. The other half of each top consisted of a hinged door made of ¼-inch weatherproof plywood. The bottoms also were made of this material. The cages were 18 inches high and 14 inches square. Each cage was provided with a pan of fine beach sand in which the grasshoppers laid their eggs.

On August 6 three cages each of *bilituratus* and *bivittatus*, collected 2 days previously on the Morrow County rangeland, were prepared. Each cage contained 10 female grasshoppers. Two of the cages for each species were replicates in which all the grasshoppers were parasitized, and the third cage contained only unparasitized grasshoppers.

On August 8 two more cages were prepared, each containing 15 *Melanoplus femur-rubrum* females collected in red clover fields of the

Willamette Valley. One cage contained unparasitized grasshoppers, and the other cage grasshoppers parasitized artificially by placement of a free first-instar *sackenii* larva upon the ventral abdominal integument. Each larva was observed until it had cut through the integument and was entirely inside the host. The parasites responded promptly and most of them succeeded in effecting complete penetration of the host in 1 to 10 minutes.

Fresh bran and such green plants as dandelion, compass plant, pigweed, lettuce, Swiss chard, red clover, and alfalfa were supplied daily. *N. sackenii* maggots were counted and removed from the cages daily as they matured and emerged from their hosts. Similar daily recordings and removals were made of any dead hosts found in the cages. The sand in the pans was sifted, and egg pods were removed and counted at intervals of approximately 2 weeks. The cages were maintained until all the grasshoppers had died from parasitism, old age, or the onset of winter.

By artificial parasitization of *femur-rubrum* it was possible to determine the developmental period of the *sackenii* larvae in the host. In the cage of parasitized *femur-rubrum* the first larva emerged on August 31, just 23 days after the host was parasitized. There was no further emergence until September 12, after which emergences followed in rapid succession until September 16. Therefore, except for the first one, emergence occurred from 35 to 39 days after the larvae had penetrated their hosts.

Effect of Parasitism on Egg Production.—Parasitism markedly reduced both longevity and egg production of hosts of the three species tested. In *bilituratus* and *femur-rubrum* curtailment of egg production was extreme and became effective in the early stage of parasitism. It appeared to be entirely independent of reduced longevity and was caused by impairment of normal egg development. In *bivittatus* curtailment of egg production was less extreme and was largely a result of reduced longevity. During their first 3 weeks in the cages, before they began to succumb from parasitism, the rate of egg production was about equal for both parasitized and unparasitized individuals. *M. bivittatus* is a much larger grasshopper than either *bilituratus* or *femur-rubrum*. Possibly this fact, as well as the fact that it is not a true host, enables it better to resist the effect of parasitism upon its repro-

FIG. 4.—*Neorhynchocephalus sackenii* fly ovipositing in cavities of old insect gall on wild rose.

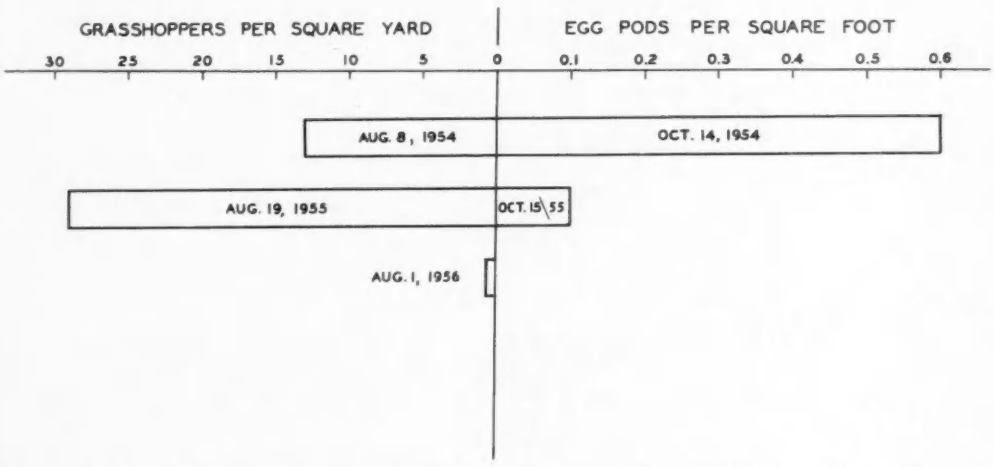

FIG. 5.—Comparison of grasshopper populations with egg deposition on rangelands in Morrow County, Oregon.

ductive capacity. A comparison of egg production of the parasitized and unparasitized hosts is shown in figure 6.

Effect of Parasitism on Host Survival.—From daily mortality records the average number of days the parasitized and unparasitized hosts survived after they were caged was determined to be as follows:

Host	Parasitized	Unparasitized
bilituratus	24	60
bivittatus	34	68
femur-rubrum	31	80

All but 2 of the 15 parasitized *femur-rubrum* had succumbed by September 15, whereas 10 of the unparasitized hosts were still alive as late as November 4.

Approximately 75 percent of the parasitized *bilituratus* succumbed during a period of accelerated *sackenii* emergence the last of August and early in September. This corresponded with the period of a sharp decline of the heavily parasitized host population on the Morrow County rangelands in 1956.

Parasite Mortality in the Host.—*N. sackenii* maggots emerged from 19 of the 20 parasitized *bilituratus* but from only 7 of the 15 parasitized *femur-rubrum*, and, as was to be expected, from none of the 20 parasitized *bivittatus* hosts. Those *femur-rubrum* grasshoppers from which maggots failed to emerge were dissected as soon as they died, and found to contain dead and sometimes badly decomposed *sackenii* larvae, often in an advanced stage of development. Such were also the findings with respect to *bivittatus*. Thus, parasitism was often fatal to the host even when the parasite itself failed to survive.

Much of the mortality of parasitized *bivittatus* and *femur-rubrum* from which *sackenii* maggots failed to emerge was associated with disease. The afflicted hosts were recognizable when alive by their flaccid and dysenteric appearance, associated with a black liquefied condition of the internal organs. Upon dissection of such hosts the *sackenii* maggots found therein were usually dead, black, and in various stages of decomposition. The contrasting healthy condition of the unparasitized hosts suggested that disease may sometimes be an important secondary effect of *sackenii* parasitism.

The lethal factors in some grasshopper species, such as *bivittatus*, that finally kill the invading parasites were not determined. All degrees of compatibility can be observed between *sackenii* larvae and the various grasshopper species that they attempt to parasitize. In *Camnula pellucida* the larvae die while they are too small to be seen with the naked eye, whereas in *bilituratus* they develop normally. In some hosts the lethal factors appear to be biochemical and in others physical in nature. In *pellucida*, for example, the larvae are able to complete the physical operations of host penetration and vent construction, but nevertheless succumb within their hosts at a very early stage. In *bivittatus* many of the larvae appear to be unable to accomplish the physical task of cutting the respiratory vent and dead larvae in the vent-cutting position are frequently visible through this host's integument. Other larvae in this host, although failing to cut a vent, develop normal respiratory tubes from the intended vent location. Such larvae attain various degrees of development, some reaching mature size, but all ultimately succumb within the host, apparently from developing resistance factors in the host's body chemistry.

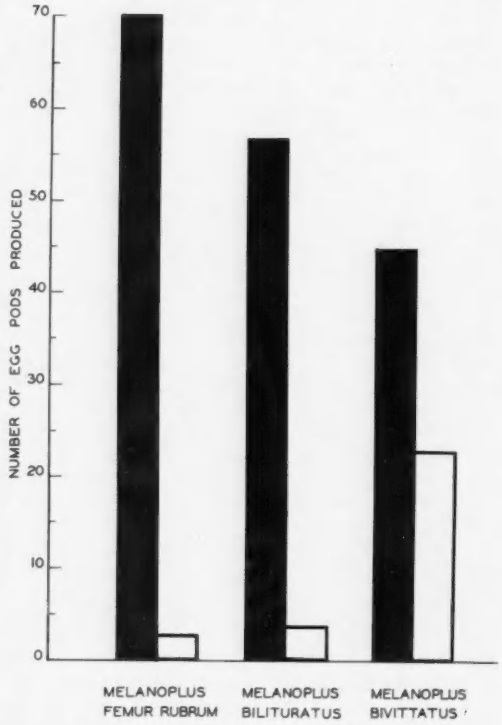

FIG. 6.—Egg-pod production of nonparasitized grasshoppers (black columns) and grasshoppers parasitized by *Neorhynchocephalus sackenii* (white columns). Based on 10 females per cage.

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APHID-VIRUS RELATIONSHIPS IN THE TRANSMISSION OF BEAN YELLOW MOSAIC VIRUS BY MYZUS PERSICAE[1,2]

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ABSTRACT

No significant differences in the virus transmission rate were found when virus acquisition feedings by the aphids were in the 11- to 45-second range, but there was a marked reduction when acquisition feedings were extended to 51 to 60 seconds. The transmission rate was higher if aphids were starved for 5 minutes or more before they fed on diseased plants. The maximum effects of preliminary starvation were obtained in 10 to 15 minutes, and no further changes in transmission rates were observed with starvation periods up to 4 hours. The differences in transmission rates between starved and unstarved aphids could not be explained by differences in acquisition feedings. Failure of some aphids to transmit the virus, while others did that had the same period of preliminary starvation, was not due to differences in the duration of acquisition feedings. Nor could large interreplicate or interexperiment variations in transmission be explained by variations in aphid behavior.

Transmission tests with the bean yellow mosaic virus by the aphid *Myzus persicae* are frequently characterized by marked variations in the rate of transmission from one replicate to another (table 1). Five organisms are involved in such transmission tests: the virus, the aphid, the plant on which the aphid develops, the diseased plant from which virus is acquired by the aphid, and the healthy plant which is inoculated. The potential sources of variation in such tests thus are numerous, particularly because of fluctuations in the greenhouse environment. As Lawrence (1957) stated, the chief physical characteristic of the average greenhouse environment is its great variability, even over relatively short periods of time. Aphids tend to feed more readily on some days than on others and, also, the first feeding puncture on the diseased plant tends to be of shorter duration on some days. Variations in feeding behavior might cause differences in the rate of transmission of a nonpersistent virus such as the bean yellow mosaic virus.

The work reported herein concerns the effects on transmission rate of variations in the length of the virus acquisition feeding and in the period of preliminary starvation preceding it. This information was needed in order to know the conditions of maximum transmission and to eliminate variation from these sources.

MATERIALS AND METHODS

The virus used was the bean yellow mosaic virus (BYMV) described by Swenson (1954). In addition to the symptoms and host range indicated in that paper, this BYMV strain also produced local lesions on *Crotalaria spectabilis* Roth. as described by Corbett (1957). Broad bean, *Vicia faba* L., was the virus source plant for the aphids. The healthy plants used as indicators of transmission were in all cases the Dwarf Horticultural variety of the garden bean, *Phaseolus vulgaris* L.

The term "acquisition feeding" refers to the feeding period on the diseased plant and in these experiments was the first naturally terminated feeding puncture or probe on the diseased plant,

[1]Approved for publication as technical paper number 1295, Oregon Agricultural Experiment Station. Accepted for publication January 15, 1960.

[2]This work was partially supported by a National Institutes of Health Research Grant and by an Oregon State College General Research Grant. The writer is indebted to Mr. S. S. Sohi for aid in performing certain experiments.

as measured from the time the tip of the proboscis touched the leaf surface until it was removed. "Preliminary starvation" designates a period of starvation after the aphid was removed from the aphid colony but before the acquisition feeding. The "test feeding" was the feeding period on the healthy plant, which is referred to as the "test plant."

TABLE 1.—Interreplicate variation in aphid transmission tests with bean yellow mosaic virus

Expt. No.	No. replicates	F	F needed for 5% level	F needed for 1% level
1	5	4.17	2.58	3.78
2	5	4.67	2.58	3.70
3	10	1.96	2.20	3.06
4	5	1.60	2.58	3.78
5	10	3.85	1.97	2.56
6	5	3.61	2.58	3.78
7	19	1.18	1.73	2.17
8	8	1.75	2.29	3.20
9	4	0.10	3.07	4.87
10	24	1.60	1.65	2.02
11	15	2.62	1.92	2.52

For the experiments represented in table 1, the aphids were starved for 15 minutes or more and given an acquisition feeding of 10–60 seconds duration. Preliminary starvation periods and acquisition feedings were variables in other experiments and are specified in connection with those experiments. All aphids remained on the healthy bean plants for at least 4 hours before removal by insecticide application.

RESULTS

Effects of variations in length of acquisition feeding.—Nonpersistent aphid-borne viruses, such as BYMV, are more efficiently transmitted if the aphids are starved for a few minutes or more before the virus acquisition feeding on the diseased plant. This is true only when acquisition feedings are kept short—a few minutes or less. The rate of transmission decreases with increasing length of the acquisition feeding. The exact point at which decrease in transmission efficiency begins is, however, seldom ascertainable from published works.

The length of the acquisition feeding period can be standardized only within certain limits because the probability of transmission is reduced if the acquisition feeding is forcibly terminated (Bradley 1952, Sylvester 1954). Transmission tests with BYMV (Swenson 1957) showed that 92 percent of the aphids, *Myzus persicae*, completed their first feeding period on the diseased broad bean plants within 10 to 60 seconds if they had been starved for 15 minutes or more. This indicated that acquisition feedings could be limited to naturally terminated feeding periods in the 10- to 60-second range with no great loss of time due to discarding the aphids which fed for longer than 60 seconds and the even smaller number which fed for less than 10 seconds.

The results of BYMV transmission tests for 1,526 aphids with naturally terminated acquisition feedings in the 11- to 60-second range following preliminary starvation of 15 minutes or more are presented in table 2. The duration of the acquisition feedings had no appreciable effect on the rate of virus transmission up to 45 or 50 seconds. Reduced efficiency occurred with periods longer than this, but this would have had no consequential effect on the results of transmission tests since relatively few aphids fed for periods of this length. Table 2 also gives the distribution of acquisition feedings in the 11- to 60-second range. The distribution of aphid feedings and the relation of acquisition feedings in the 11- to 60-second range to transmission rate are in essential agreement with the results obtained by Bradley (1954) for transmission of potato virus Y by *Myzus persicae*. Bradley's paper is the only work presenting sufficient data to allow comparisons of this nature.

Further evidence that variations in the length of acquisition feedings in the 11- to 60-second range do not result in detectable variations in the transmission rate was obtained in an experiment with 360 aphids. BYMV was transmitted by 126 of these aphids; 234 of them did not transmit the virus. The mean acquisition feeding for the 126 transmitters was 24.1 seconds; that for the 234 nontransmitters was 23.9 seconds.

Effect of preliminary starvation.—Almost the full effects of preliminary starvation can be ob-

TABLE 2.—Relation of length of virus acquisition feeding to the rate of BYMV transmission by the aphid, *Myzus persicae*.

	Acquisition feeding (in seconds)									
	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60
Number aphids fed	265	344	301	192	129	90	58	53	39	55
Percent aphids	17.4	22.5	19.7	12.6	8.4	5.9	3.8	3.5	2.6	3.6
Number transmitted	85	122	108	73	38	30	19	15	10	12
Percent transmission	32.1	35.5	35.9	38.0	29.5	33.3	32.8	28.3	25.6	21.8

TABLE 3.—Relation of length of preliminary starvation period to bean yellow mosaic virus transmission by *Myzus persicae*.

Expt. No.	Minutes of preliminary starvation[a]						Based on[b]
	0	4–7	14–20	30–40	60–75	120–135	
1	12	—	33	42	21	25	24
2	8	38	44	36	26	—	50
3	—	28	52	62	36	50	50
4	—	—	54	48	62	54	50
5	—	—	50	60	52	54	50
Mean (%)	9.6	33.0	48.2	50.4	41.5	49.0	
	10	*20*	*30*	*40*	*50*	*60*	
6	59	50	43	57	57	46	56

[a]Results given as percent plants infected.
[b]Number of plants per starvation period.

tained in 15 minutes with some nonpersistent viruses, whereas with others transmission efficiency increases slowly, reaching a maximum after several hours (Sylvester 1954).

The results of six experiments concerning the effects of different periods of starvation on BYMV transmission by *Myzus persicae* are presented in table 3. The acquisition feedings were of 10 to 60 seconds duration in experiments 1, 2, and 3, and 10 to 45 seconds duration in experiments 4, 5, and 6. The treatments (starvation periods) were made in random order in experiments 1, 2, 3 and 6; in experiments 4 and 5 aphids representing the various starvation periods were fed on diseased plants and transferred to healthy plants in order of increasing length of the starvation periods. Because of the variability in the results obtained from experiments 1–5, experiment 6 was made using more narrowly defined starvation periods. The results of this last experiment differed neither in being characterized by less variation nor in the general pattern of transmission obtained for the different starvation periods.

For BYMV transmission by *M. persicae* the maximum effects of preliminary starvation appear to be obtainable in 10–15 minutes with a considerable part of the effects manifested at the end of the first 5 minutes. Effects of prolonging the starvation period to 4 hours were tested in two experiments. The rate of transmission following 4 hours of preliminary starvation was not significantly different from that after 2 hours.

The first feeding period (as defined herein) of nonstarved aphids tended to be longer than that of aphids which had received a period of preliminary starvation. This observation led to the recording of acquisition feedings of all aphids used in one of the experiments on the effects of starvation (number 2, table 3) to learn if any part of the effects of starvation could be explained by variations in the acquisition feedings. Length of acquisition feeding decreased sharply from 37.8 seconds for nonstarved aphids to 23.9 seconds for aphids which had been starved for 30–40 minutes (table 4). There was no relationship between the number of plants infected and the length of the acquisition feedings for the different periods of preliminary starvation. Furthermore, for each of these periods there were no significant differences between the mean acquisition feedings of the aphids which transmitted the virus and those which did not (table 4). For the entire experiment, the mean acquisition feeding of the 76 aphids which transmitted the virus was 28.5 seconds and that of the 174 aphids which did not transmit it was 30.4 seconds.

TABLE 4.—Relation of acquisition feeding to length of preliminary starvation period

	Minutes of starvation				
	0	4–7	14–20	30–40	60–75
Plants infected (percent)	8	38	44	36	26
Mean AF[a]—All aphids	37.8	34.1	29.3	23.9	24.3
Transmitters	35.5	34.6	27.8	24.1	25.0
Nontransmitters	38.0	33.7	30.4	23.8	24.1

[a]In seconds.

DISCUSSION

The results of these experiments indicate that the maximum transmission occurs with acquisition feedings of 11 to 45 seconds duration. There are no apparent differences within this range. Maximum effects of preliminary starvation in

increasing transmission were obtained in 15 minutes. The replicates in the 11 experiments represented in table 1 generally were inoculated on successive days. All the transmission tests were made with aphids which had been starved for at least 15 minutes and then fed on the diseased plants for 10 to 60 seconds. The large day-to-day variation in transmission rate thus could not possibly have been due to variations in preliminary starvation periods and acquisition feedings.

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ACTIVITY AND ABUNDANCE IN INSECT LIGHT TRAP SAMPLING[1]

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ABSTRACT

A method is presented for the calculation of the theoretical catch level of insects taken in a light trap or other sampling device. Departures from the theoretical catch are due to high or low activity, and must be accounted for by weather. Weather is defined as the total pattern of all factors of the physical environment. Several methods of calculation for both the population curve and the activity coefficient are developed and tested in a multiple regression calculation based on the log of the minimum temperature and the log of the minimum relative humidity. In terms of this comparison the most nearly correct population curve was found to be that derived from catch points whose weather patterns were closest to the mean weather pattern for all points available, and the best activity calculation resulted from the relationship

$$A = \frac{\log \text{catch}}{\log \text{population}}.$$

Williams (1940) was one of the first to express with mathematical logic a theory and analysis of light trap sampling for the determination of insect abundance and activity, and to demonstrate the fact that any given catch is a resultant of two factors: the activity of a population at a particular time, and the size of the population available for sampling. These factors are related in the simple expression

(1) $C \propto AP$

where C is the catch, A the activity, and P the population or abundance. If the efficiency of the trap does not change, any change in the actual abundance, P, will be reflected in the P component of the catch, and the expression becomes an equality

(2) $C = AP$

where P is now not the population level as such, but that part of the catch which is the result of the population level.

In another paper (1937) the same author demonstrated the desirability of logarithmic transformations for data of this kind, and the basic equation becomes

(3) $\log C = \log A + \log P.$

Log A, in turn, is the aggregate of the weather components, since the flight response of the available population is almost entirely the result of current weather. It may be expressed as

(4) $\log A = (\log \text{activity due to } W_1 + \log \text{activity due to } W_2 + \ldots \log \text{activity due to } W_n)$

for the total of all physical factors acting on the population, and the log catch is thus seen to be composed of "portions due to the effect on activity of the different environmental factors plus a portion determined by the total population available." Theoretically, and with due allowance for random error, the total variation of activity should be entirely accounted for by the proper recording and evaluation of the total weather pattern during the time of flight.

Two formidable difficulties block the path to any such complete analysis; the first theoretical, the second practical. First, an adequate method must be devised for separating the A and P components of the catch, in order that that which is to be evaluated can be defined; second, the environmental factors which affect activity may number a dozen or more; instruments to record

[1]Accepted for publication January 25, 1960.

them are expensive, delicate, and in some cases not on the commercial market; and once such voluminous data have been gathered they can hardly be processed without access to electronic computing facilities.

The present paper is addressed to the problem of defining the activity component in catch data, and is illustrated with information on the first native generation of the cotton leafworm (*Alabama argillacea* Hbn.) at Florence, South Carolina, in 1957.

The need for some definition of activity was fully appreciated by Williams in 1940. His solution of the difficulty was to work with catch differences on successive nights, under the assumption that the change in the population level in such a short period of time was negligible. This assumption is quite valid when one is measuring, as he did, the collective behavior of many insect species and when the total population changes but little from day to day. It is less so when one considers the population of a single generation of one species.

Adult emergence in an insect population, like any other biological event, will occur with few exceptions in a random manner and result, for a single generation, in a "normal curve" type of distribution when plotted against time. The height of the curve will depend on the size of the population and on the length of life of the adults. The standard deviation will depend on the extent to which optimum conditions prevailed during and before the period of emergence and again on adult longevity. The curve is thus seen to be not "normal" in the strict statistical sense; it is symmetrical, and, as adult longevity decreases, it approaches on each side a sigmoid curve, or ogive, whose equation is

$$(5) \quad Y = Ce^{-\frac{(M-X)^2}{2\sigma^2}}$$

where M is the mean of the curve and C is its height or mode. (Note that C has previously been used in this paper to denote catch.)

Forbes (1953) noted that, for computations of the kind which follow, a more satisfactory expression of the same equation is

$$(6) \quad \log Y = -k \log e\,(M-X)^2 + \log C$$

where $k = \frac{1}{2\sigma^2}$.

Equation (6) above contains three unknowns: the mode, the mean, and the standard deviation of the population curve to be plotted. A provisional mean is midway between the first and last catches in a single-generation period, or in some cases it may be possible to estimate the mean from known facts in the biology of the species under consideration. Given M, and two or more points on the population curve, it is possible to calculate k and log C in (6) from two simultaneous equations derived as follows:

According to the method of least squares the best fit for a set of data is that curve for which the sum of the squares of the residuals is a minimum. To obtain this best fit for a set of data (X_i, Y_i), $i = 1, 2, \ldots n$, where n represents the number of observations, a function f(C, k) is formed by using the residuals $(Y-Y_i)$ where Y and Y_i are, respectively, the values from the curve of best fit and the observed values corresponding to X_i. The function f(C, k) is defined by

$$f(C, k) = \sum_{i=1}^{n} [\log C - k(M-X_i)^2 \log e - \log Y_i]^2$$

This function will be a minimum for those values of k and log C which cause the partial derivatives for the function with respect to k and C to be zero simultaneously. The partial derivatives set equal to zero are:

$$\frac{\partial f}{\partial C} = \sum_{i=1}^{n} 2[\log C - k(M-X_i)^2 \log e - \log Y_i]\frac{\log e}{C} = 0$$

$$\frac{\partial f}{\partial C} = \sum_{i=1}^{n} 2\,[\log C - k(M-X_i)^2 \log e - \log Y_i]$$
$$[-(M-X_i)^2 \log e] = 0$$

Upon simplification these simultaneous equations in k and log C then become

$$(7) \quad n \log C - k \log e \sum_{i=1}^{n} (M-X_i)^2 = \sum_{i=1}^{n} \log Y_i$$

and

$$(8) \quad \sum_{i=1}^{n} (M-X_i)^2 \log C - k \log e \sum_{i=1}^{n} (M-X_i)^4 = \sum_{i=1}^{n} (M-X_i)^2 \log Y_i$$

The P curve thus obtained from equations (6), (7), and (8) is an approximation of the P component of the catch (see equation (2)), if the adult longevity is the same as the time interval of sampling. It is an "expected catch"—the catch that would have been obtained had all activity been held constant. Before going further, attention must be called to its limitations, sources of error, and a correction for adult longevity.

Unless good information on the life history of the insect concerned is available, the mean must be selected somewhat arbitrarily. A mean must be selected, since M is a necessary factor in the calculation. This difficulty can be overcome only by selecting a provisional mean, working the problem through to the point of obtaining a partial regression on a certain weather factor, and then repeating the process with another trial mean until the regression is at its highest point.

The P curve depends for its mode and standard deviation on the two or more points selected from which to calculate it. No matter how carefully they are examined, these points, being catch, are still contaminated with activity. In the present state of our knowledge there is little that can be done to adjust these points, with reference to their respective associated weather patterns, to

equalize the activity effect between them. Yet two days on which even the most significant weather factors are identical are most unlikely within the span of a single generation. Three alternatives are possible here, all of which have been investigated: (1) The weather factors available may be averaged, separately, and the key points selected from those catches whose corresponding weather factors most closely approach the average; (2) Logs of all available catch points may be summed in $\Sigma \log Y_i$, on the assumption that variables in the weather pattern will tend to equalize each other; (3) The key points may be taken from those days having the most nearly identical weather factors. Comparison of these three techniques will be presented in the example which follows.

TABLE 1.—Relationship between mode of population and length of adult life.

If D is	Height of mode is
1	C
2	$C+C_1$
3	$C+2C_1$
4	$C+2C_1+C_2$
5	$C+2C_1+2C_2$
6	$C+2C_1+2C_2+C_3$
n(even)	$C+2C_1+\ldots+\ldots+\ldots C_{\left(\frac{n}{2}\right)}$
n(odd)	$C+2C_1+\ldots+\ldots+\ldots 2C_{\left(\frac{n-1}{2}\right)}$

The population curve represented by the calculated constants must be corrected for the cumulative effect caused by the length of life of the adults. If all adults died the night they emerged—i.e., were only exposed to the trap once—the rise and fall of the generation would be represented by the line already calculated. A more realistic model, however, demands that the individuals which emerge the first night be added to those which emerge the second, etc.

The mean of the population curve moves later in time by the amount $[\frac{1}{2}D-\frac{1}{2}]$ days, where D is the assumed average length of life. The mode increases in height in a manner indicated in table 1. In this table, D is the average length of adult life in days, C is the height of the original mode, from equations (7) and (8), and C_1, C_2, C_3, . . . C_n are the successive Y values calculated from these equations adjacent to C. The value of σ also increases as the assumed length of life increases.

The result of this additive correction is that the corrected curve no longer corresponds to any series of points that can be computed by the use of the sigmoid curve equation (6). Nevertheless, its value as a working tool has been somewhat increased.

To return to the method. The calculated and corrected P curve is to be compared with the catch in such a way as to resolve the two curves into a single series of points. Also it is desirable to transform the positive and negative deviations of the catch curve from the P curve to logarithms. These deviations are a measure of the total activity, and again several choices of method are possible. The simplest is to transform both series of points to logs immediately, and divide log catch by log P to give a series of activity constants. For purposes of analysis of variance, the total sum of squares in this case is $\Sigma (Y-1.00)^2$, where Y is the activity constant. To avoid repetition in the discussion of the example, this process will be referred to as method I.

A second technique, designated hereafter as method II, is to divide the catch by the population values, record each quotient as a deviation from 1.00 (since 1.00 represents "normal" activity), add 1 to avoid negative logarithms, and then convert to logs. Each term must carry a + or − sign after this conversion, to indicate the direction of the deviation whose magnitude is now expressed logarithmically. The total sum of squares in this case is ΣY^2.

It is possible also to consider activity as varying in degree from some optimum activity brought about by the best possible combination of weather factors. Such a situation probably never in fact occurs, yet a population model constructed on optimum conditions, could they be derived, would have many uses. As a start in this direction, activity may be calculated as in method I above, then tabulated as deviations from the highest activity displayed. The total sum of squares is as in method II. For proper analysis by this method the optimum level for each weather factor must be known or assumed, and the correlations are made with the weather components also expressed in terms of deviations from their respective optima. An analysis on this basis is at present little more than hopeful speculation, but for purposes of comparison it is presented below as method III.

The statistical techniques by which the extent of the influence of one or more weather factors on activity may be measured are available from many sources and need no elaboration here. It is not the purpose of this paper to undertake a comprehensive analysis of the effect of weather factors on activity, or to inquire into the statistical significance or nonsignificance of any of them. It is sufficient to submit the different choices and possibilities noted above to some standardized process by which they may be compared and evaluated, in order that some opinion may be formed regarding the value of the different approaches. Two weather variables were selected: the log of the minimum temperature recorded during the night, and the log of the minimum relative humidity recorded during the

TABLE 2.—Constants for calculation of P curve based on three different criteria.

	All catch points	Two points with T_{min} and RH_{min} closest to means	Two points with T_{min} and RH_{min} most nearly identical
$\Sigma(M-X)^2$	1,785	205	181
$\Sigma(M-X)^4$	327,369	29,857	16,561
Σlog Yi	47.387	5.585	4.534
n	18	2	2
Σlog Yi$(M-X)^2$	3723.976	520.001	406.337
$\Sigma[(M-X^2)]$ log e	774.690	88.970	78.554
$\Sigma[(M-X^4)]$ log e	142,078.146	12,957.938	7187.474
log C	3.27717	3.40048	4.26753
C	1,893	2,515	18,515
k	0.01495	0.01367	0.05093
k log e	0.00649	0.00593	0.02210
R	0.18	0.27	0.15

preceeding day. The multiple regression coefficient R, which in this case indicates the degree of association between the dependent variable (activity) and both of the two independent variables, was calculated in each step of the example, and the size of each of the Rs is a measure of the efficiency of the particular method that produced it. If one's primary interest is in data rather than in method, an extension of the above process to include an analysis of variance would be appropriate.

EXAMPLE

The cotton leafworm (*Alabama argillacea* Hbn.) does not winter in South Carolina; it spreads northward in successive generations every summer and arrives at Florence, S. C., about the middle of August. Food is usually available, and this insect is known to mature from egg to adult in about 18 days. The length of adult life in South Carolina is not known; Hull (1929) in Texas reported little mortality in 3 days, and a maximum life of 11 days, without food. We will assume 3 days in the calculations which follow. The complexities introduced by migration and overlapping generations make difficult an analysis for this insect beyond the first peak of emergence; moreover, for the purpose of illustration intended here such an extended analysis is not necessary. Therefore only the rising curve of the first native generation, which started on September 2, 1957, will be used.

The tendency of this insect to spread north introduces a complication into an attempt to set up a theoretical model. From approximately August 17 to approximately September 2 there were no native adults at this locality. There was presumably more spread in from the south during this time than there was out toward the north, and the presumed net increase of adults due to spread continued through the flight period of the native adults. If this is indeed the fact, it is obscured by activity; a regression line fitted to the logs of the catches from August 17 to September 2 has a slightly *negative* slope, indicating a net *loss* of adults during this time.

CALCULATION OF P CURVE

Table 2 presents the constants and multiple regression coefficients resulting from the calculation of the fundamental P curve in three different ways. These calculations assume that the greatest number of adults emerged on September 19—18 days after the start of the emergence of the first native adults. The three columns of the table are constants resulting from, respectively, (1) all catch points for the 18 days, (2) the use of two points (September 6 and 13) whose RH_{min} and T_{min} most closely approach their average values, and (3) two points (September 9 and 10) whose RH_{min} and T_{min} are most nearly identical. Fair agreement exists between C and k in the first two instances; the great discrepancy between the third column and the other two is

TABLE 3.—Corrected P curve and catch.

Date Sept.	Y	Y_c	log Y_c	Catch	log catch
2	49	49	1.687	18	1.255
3	76	125	2.097	80	1.903
4	116	241	2.382	66	1.819
5	173	365	2.562	126	2.100
6	250	539	2.731	208	2.318
7	352	775	2.890	292	2.465
8	482	1084	3.035	448	2.651
9	642	1476	3.196	114	2.057
10	832	1956	3.291	300	2.477
11	1050	2524	3.402	384	2.584
12	1288	3170	3.501	444	2.647
13	1358	3696	3.588	1848	3.267
14	1778	4424	3.664	656	2.817
15	2021	5157	3.728	810	2.908
16	2224	6023	3.771	4542	3.657
17	2381	6626	3.821	2370	3.375
18	2481	7086	3.850	2353	3.371
19	2515	7377	3.868	5200	3.716
20		7477	3.874	2176	3.338

doubtless due in part to the fact that RH_{min} and T_{min} were not identical on September 9 and 10, and the variation of both factors was in favor of greater activity on the 10th.

When the multiple regression coefficient R is calculated for each of the three P curves by the method of $\frac{\log \text{ catch}}{\log \text{ P}}$ it is found that the calculation based on the selection of two points whose independent variables are closest to their own means yields the highest regression. The constants of the second column in table 2, therefore, were used in computing the P curve for further work.

TABLE 4.—Activity constants computed by three different methods.

Method I	Method II	Method III
0.744	−0.213	0.226
0.907	−0.134	0.063
0.764	−0.237	0.206
0.820	−0.219	0.150
0.849	−0.208	0.121
0.853	−0.210	0.117
0.873	−0.201	0.097
0.644	−0.284	0.326
0.753	−0.266	0.217
0.760	−0.267	0.210
0.756	−0.270	0.214
0.911	−0.176	0.059
0.769	−0.268	0.201
0.780	−0.266	0.190
0.970	−0.096	0.000
0.883	−0.215	0.087
0.876	−0.222	0.094
0.961	−0.112	0.009
0.862	−0.233	0.108
R 0.325	0.198	0.064

The methods on which the constants in column 3 are based, i.e. selecting two points having identical weather conditions, is theoretically sound and should not be discarded, but the difficulty of employing it in practice is well illustrated here.

CORRECTION FOR LENGTH OF LIFE

The P curve selected and calculated from the constants in table 2 suffers from the deficiencies already discussed. Its points (Y) are given as the second column of table 3. If adults were available for trapping for an average of three days after emergence, the corrected P curve, Y_c, is the third column of table 3. Note that it contains one more item, since the three-day accumulation moves the mean one day. The mode has also increased in height in the manner indicated in table 1. This, then, is an approximation of the P component of the catch referred to in equation (2). Its logs make up the fourth column of table 3.

CALCULATION OF ACTIVITY

There remain to be considered the three methods of determining the activity coefficients for the series of catches. Their methods of derivation are explained above, and designated as I, II, and III, respectively. Columns 5 and 6 of table 3 are actual catch and log catch for the 19 nights. Table 4 gives the three series of activity coefficients, with the R obtained by completing the calculation with each. It would appear that the greatest accuracy in determining activity is attained by use of method I.

The independent variables, taken as logs of the minimum temperature and the minimum relative humidity of the previous day, are given in table 5, together with their deviations from presumed optimum levels for the regression by method III. One can assume that the minimum temperature, which varied for these 19 nights between 73° F. and 66° F., was never so high as to be detrimental to activity; hence the deviations in the third column of table 5 are departures from the log of the highest minimum temperature. On the other hand, it is possible that there is an optimum RH_{min}, and that both higher and lower values of this factor depress activity. There is no evidence that such an optimum exists for this insect, or, if it does exist, that other values of RH_{min} are randomly distributed with respect to activity; the reasoning here is purely pragmatic. Therefore column 4 of table 5 was calculated as deviations of log RH_{min} from the log RH_{min} which occurred the day before the night of highest activity, September 16. The fact that columns 3 and 4 are both in terms of deviations from the same log (1.863) is merely a coincidence.

TABLE 5.—The independent variables.

Date Sept.	log T_{min}	log RH_{min}	log T_{min} as Dev. from 1.863	log RH_{min} as Dev. from 1.863
2	1.857	1.681	0.006	0.182
3	1.845	1.681	0.018	0.182
4	1.857	1.708	0.006	0.155
5	1.826	1.690	0.037	0.173
6	1.839	1.813	0.024	0.050
7	1.839	1.903	0.024	0.040
8	1.845	1.968	0.018	0.105
9	1.833	1.851	0.030	0.012
10	1.839	1.857	0.024	0.006
11	1.857	1.778	0.006	0.085
12	1.851	1.770	0.012	0.093
13	1.839	1.778	0.024	0.085
14	1.833	1.778	0.030	0.085
15	1.820	1.770	0.043	0.093
16	1.839	1.863	0.024	0.000
17	1.857	1.851	0.006	0.012
18	1.863	1.914	0.000	0.051
19	1.839	1.954	0.024	0.091
20	1.833	1.778	0.030	0.085

CONCLUSIONS

If the assumptions presented regarding the emergence of an adult population are valid, it appears possible to reconstruct a curve which represents population behavior, and from it calculate the activity component for any given catch. The activity may then be used in further analysis for weather effects, trap comparisons, etc. Of the methods attempted for the calculation of the population curve, one based on the selection of two points having identical weather patterns is theoretically the most accurate, but such a fortuitous combination of weather factors is unlikely. The next best alternative is the use of points whose weather factors most nearly approach their own means. Of the methods of obtaining the activity coefficient, the division of $\text{logs} \frac{\log \text{catch}}{\log P}$ appears preferable.

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OLFACTOMETER AND PROCEDURE FOR TESTING BAITS AND CHEMICAL ATTRACTANTS AGAINST HIPPELATES EYE GNATS[1]

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ABSTRACT

A simple new trap and olfactometer have been designed for screening and evaluating baits and chemical attractants against *Hippelates* eye gnats. The system has proved equally efficient against *Milichiella* species and several other small dipterous flies. The design of the trap was based on the phototropic and geotropic responses and olfactory behavior of the eye gnats. Various factors such as the lighting level of the funnel surface area and the depth of different types of bait dishes were found to influence the efficiency of the trap markedly. Increase in the lighting level in the funnel increased the entry of the gnats into the trap and the collection of gnats in the vial. Similarly, the use of shallower bait dishes having greater surface areas resulted in larger collections of gnats in the traps. The presence of an insecticide in the collection vial of the trap increased efficiency, and a number of insecticides were screened for quick knock-down and low repellency. Traps containing toxicants such as malathion, parathion, dicapthon, Dylox®, Korlan® and others gave larger collection of gnats than traps without insecticides in the vials.

No marked difference in the gnat collections in the traps was observed when window screen (16 mesh) or hardware cloth (8 mesh) was used as trap support on a turntable in the open shade. However, traps having the coarser mesh screen support captured larger numbers of gnats than the traps with the finer mesh screen support and caught significantly higher numbers of gnats in the bait dish when the experiment was run in the open sun. The selection of one type of screen rather than another depends largely on the conditions under which experiments are to be performed.

Pestiferous species of the genus *Hippelates* are greatly attracted to man and animals. They feed mainly on the moist surfaces of the eyes, ears, nose, and mouth of their host, and they alight and feed on other exposed parts of their host's body. Lacerations, cuts, and wounds are also sites of greater feeding activity of these pests. It is believed that this attraction behavior is initially triggered by olfactory stimuli, chemical in nature, emanating from the host. It is possible that physical factors such as color, movement, shape, and thermal and moisture gradients associated with the hosts also play an important role in the attraction behavior of eye gnats.

The problem of understanding the chemical nature of the various olfactory stimuli that draw eye gnats from a distance to their hosts and to other materials has been considered of importance for a long time. However, due to the lack of a suitable device for measuring the response of eye gnats to these stimuli accurately and quantitatively, this phase of the work has not been investigated to any extent.

During the past three decades various kinds of bait traps have been constructed and designed for trapping eye gnats either for reducing gnat populations or for measuring gnat populations, but none of these traps has been considered

[1]Paper No. 1199, University of California Citrus Experiment Station, Riverside, California. Studies conducted under a grant-in-aid from the Coachella Valley Mosquito Abatement District, Riverside County, California. Accepted for publication February, 1960.

suitable for critical comparative testing of small analytical samples of chemicals.

Parman (1932a, 1932b) designed bait traps for the specific purpose of capturing large numbers of eye gnats in the Coachella Valley. These traps were made of wood and glass panel and were 48 inches wide, 74 inches long, and 103 inches high. Burgess (1935, 1951) modified the Parman box-type bait trap and designed many other simple types of bait traps for use in the program for abating eye gnats around residences and schools, as well as for indexing seasonal population fluctuations.

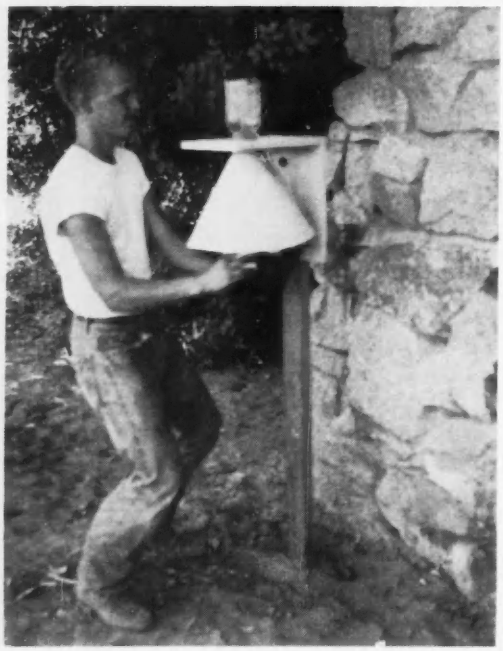

FIG. 1.—Tinkham eye-gnat bait trap used in measuring adult eye-gnat populations.

More recently a new type of bait trap was designed by Tinkham (1949) for measuring adult eye-gnat populations. This trap (fig. 1) seems to be the most versatile of all the eye-gnat traps available to date and is currently employed in measuring adult eye-gnat populations in California. Further improvements in the size and construction of eye-gnat bait traps were investigated by Dow and Hutson (1958) and by Dow (1959), who developed a relatively simple metal trap, using a 1-pint glass fruit jar as a collection chamber. This simplified trap, according to the authors, eliminated many of the objections inherent in the Burgess box-type trap.

Since the traps employed thus far in collecting and capturing *Hippelates* eye gnats were considered to be inadequate for critical studies of attractants, it became necessary to investigate the development of a suitable trap or olfactometer for this purpose. An efficient small bait trap was developed and tested extensively. The trap has not only proved efficient in attractant studies against eye gnats, but has also provided information on the attractants of *Milichiella* species and several other small dipterous species. Several of these traps, when used on a screen frame or a turntable as a set, form what is essentially an olfactometer by which various materials or different concentrations of the same material can be evaluated. This testing unit has been named the C. E. S. (Citrus Experiment Station) olfactometer and it meets the requirements and features set forth for an efficient testing unit to be used in exploring eye-gnat attractants.

The olfactometer has yielded excellent results in studies against the four species of *Hippelates* eye gnats encountered in southern California thus far. These are *H. collusor* (Townsend), *H. robertsoni* Sabrosky, *H. dorsalis* Loew, and *H. pusio* Loew. The experiments reported here, however, were performed in the Coachella Valley and other areas of southern California where *H. collusor* is the predominant species. Very few individuals of the other species were captured in the traps, and these for the sake of simplicity were included in the count of *H. collusor*; and the data presented in the tables refer to the numbers of this species.

ESSENTIAL FEATURES OF AN EYE-GNAT TRAP AND OLFACTOMETER

A suitable trap or olfactometer to be used in screening attractants in the field against eye gnats should have the following features:

1) The apparatus should be simple in both size and design. This makes it economically feasible to build large numbers of these necessary for adequate replication. Small traps, in addition to being more economical than large ones, are easily transported and can be set out in a short period of time.

2) The individual traps or units of an olfactometer should be subjected to thorough washing after use. This operation removes traces of materials tested previously with this equipment. Items made of glass are therefore essential for constructing the main parts of the trap or olfactometer.

3) The olfactometer should yield reproducible results with the least possible variation.

4) Replication of the traps or units of an olfactometer should be possible in a limited, homogeneous environment. In this regard small-sized traps are more desirable than larger units. It is necessary to equalize the effect of environment and the directional flight tendency of gnats by some other means.

5) Once an experiment is in progress, the olfactometer should be flexible enough to be

moved short distances without being disassembled. Frequently this shifting of position becomes necessary for movement from an unfavorable gnat niche to one where gnats are more active.

6) It should be possible to test microquantities of materials in the device.

7) The unit should yield results in a short period of time, that is, in 2 to 3 hours. Units that require longer periods of time are not desirable. Such units not only slow down the screening operation but also affect the attractancy of the materials tested, owing to the presence of captured gnats in the traps which attract more gnats.

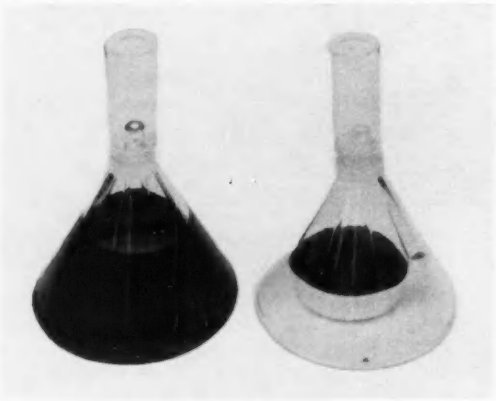

FIG. 2.—C.E.S. eye-gnat trap with bait dish, one funnel partially painted black.

C.E.S. EYE-GNAT OLFACTOMETER[2]

This olfactometer consists of four main parts, as described below.

1) *C.E.S. Eye-Gnat Trap.*—This trap is made of a 100-mm. Pyrex fluted clear funnel with a 7-dram display glass vial as a collection chamber (fig. 2). The stem of the funnel is cut off about 10 mm. from the neck. The outer circular edge of the cut stem is ground and tapered slightly for safety reasons. This also facilitates easy fitting of the collection vial closure over the stem of the funnel.

The collection vial is fitted with a polyethylene closure from which the hard, inelastic supporting disk is removed. A small hole equal to the outer diameter of the stem of the funnel is drilled in the center of the closure. The closure can be fitted easily into the vial, and the collection vial can then be inverted and slipped over the stem of the funnel. The polyethylene closure fits tightly over the funnel a few millimeters below the neck line.

[2]The assistance of Rowland Maddox of the Citrus Experiment Station in constructing the turntable and the housing for the reductor, motor, and speed-control units is greatly appreciated.

2) *Bait Dish.*—The bait dish is placed under the trap over the screened portion of the olfactometer. The type and size of the dish were found to influence the efficiency of the trap (see below). Round aluminum-foil dishes (diameter, 57 mm., depth, 18 mm.) have been used, since these can be discarded after use.

3) *Turntable.*—The turntable (fig. 3) is made of high-grade exterior plywood ¼-inch thick. It is circular in shape with a diameter of 4 feet. A 7-inch turntable disk is fastened in the center of the turntable; a hole in the center of the disk with a set screw accomodates the ½-inch vertical shaft from the reductor.

Twenty circular holes, 2 inches apart, are cut into the turntable periphery. The diameter of the hole is equal to the outer diameter of the funnel so that the latter can be inverted over the hole and the screen support, as described below.

Most of the materials tested in this olfactometer proved to be attractant to both house flies and blowflies. A provision for excluding these larger insects has been incorporated into the device so that only eye gnats and gnat-like insects may be captured. This makes the final count and segregation of eye gnats easier.

Preliminary observations have indicated eye gnats to be attracted to traps where house-fly activity is great. By excluding house flies and similar, large-sized insects, this interference problem is largely overcome.

Pieces of household window screen (16-mesh) or hardware cloth (8-mesh) are stapled over the holes on the underside. These screen disks act as supports for the funnel trap and also keep insects that are much larger in size than the eye gnats from entering the trap. Coarser meshes of hardware cloth do not exclude house flies and similar insects which may be attracted to the test materials. The gnats easily pass through the screen and into the trap. No leaks or spaces can develop between the screen and the edge of the funnel trap to permit entry from above the screen disk. If larger insects are to be admitted into the trap, coarser mesh screen disks can be used for trap supports.

Electric current is required for operating the turntable. Where the current is not available, the C.E.S. eye-gnat traps can be arranged on screen frames of any design. The screen can be stapled to round, square, or rectangular frames having folding or removable legs 2 to 3 feet high. The frames should not have surface areas of more than 6 to 8 square feet. Frames larger than this sag toward the center and this results in incomplete coverage of the bottom of the dish by the material that is being tested. In comparison, the same amount of liquid will cover the whole surface of the bait dishes when these are located near the middle portion of the frame where surface inclination is at a minimum. Larger screen frames also have the disadvantage of progressive

FIG. 3.—C.E.S. eye-gnat olfactometer assembly, as used in screening chemical attractants and baits against eye gnats in the field.

reduction of gnat collection from peripheral traps to traps closer to the center. The screen frames are stationary and can be rotated manually at regular intervals.

4) *Power Unit, Speed Controller, and Reductor.*—The housing below the turntable (fig. 3) contains an electric motor (1/20 horsepower, 1725 rpm), a Zero-Max variable speed torque converter (Model 14, type CW, with 10-inch lbs. torque and speed range 0–400. Revco, Inc., Minneapolis, Minnesota) and a Boston gear reductor (Ratio, 100:1, type, VLW13, Boston Gear Works, Quincy, Massachusetts). The motor is coupled with the Zero-Max through a central flexible coupling (Central Die Casting and Mfg. Company, Chicago, Illinois) and the latter is joined to the input shaft of the gear reductor through a similar arrangement. An 18-inch aluminum rod ½ inch in diameter is coupled with the output shaft of the gear reductor. The aluminum rod acts as a support and shaft for the turntable.

EXPERIMENTAL PROCEDURE

To assemble the C.E.S. eye-gnat olfactometer, the turntable is slipped over the aluminum rod protruding above the housing, the set screw is tightened, and the speed of the turntable is then determined. One revolution of the turntable in 4.0 minutes has been found to be satisfactory.

The aluminum bait dishes are set on the screen disks, and liquid or solid attractants are placed in these dishes in the desired quantities. Once attractant materials are placed in the dishes, the turntable is kept turning until the experiment is terminated.

It has been observed that gnats entering the collection vial will occasionally escape back through the stem of the funnel. To overcome this difficulty, some quick knock-down insecticide is used in the vials. Throughout these studies, 0.5 ml. of 0.02% dicapthon in acetone was added per vial and the solvent evaporated.

After addition of the test materials to all the dishes, the C.E.S. eye-gnat trap is inverted over the bait dish. The experiment is continued until sufficient numbers of eye gnats are captured in the traps containing standard materials. The experiment is then terminated and the small holes in the closures of the collection vials are stoppered to prevent live gnats from escaping. Whole egg powder (0.1% to 0.5%) aged for 5 days at 90° F. has been employed as the standard attractant.

All the components of the C.E.S. eye-gnat trap should be thoroughly washed with soap and warm water before they are reused. The collection vials are again provided with the insecticide. In transporting the funnels, breakage can be avoided by fitting over the stem of each funnel a rubber stopper in which a hole has been drilled. The funnels can be stacked and placed in cardboard boxes and moved from field to laboratory and vice versa.

C.E.S. Eye-Gnat Trap versus Tinkham Bait Trap.—The efficiency of the C.E.S. eye-gnat trap was compared with that of the Tinkham bait trap. The latter, as described above, is currently used for indexing adult gnat population prevalence. The trap is relatively simple and is suitable for measuring long-term population trends.

In one test, pairs of both types of traps were tested at 2- and 3.5-foot levels above ground. No significant difference was observed between catches at the two types of traps at either level (table 1).

TABLE 1.—Comparison of collection of C.E.S. and Tinkham eye-gnat traps at two different levels above ground[a]

Type of trap	Level above ground (feet)	Average No. of gnats		Total
		Bait dish	Vial or jar	
Tinkham	2.0	85	169	254
Tinkham	3.5	91	215	306
C.E.S.	2.0	129	124	253
C.E.S.	3.5	77	233	310
Statistical significance[b]		N.S.	N.S.	

[a]Five replicates, two pairs of traps (one of each kind at each level) on each stand and ¼ oz. egg bait was used as the attractant. The egg bait was obtained from bait traps used for indexing gnat prevalence.

[b]Data transformed to $y=\sqrt{x}$ and analyzed for variance.

Stationary Screen Frames versus Turntables.—The C.E.S. eye-gnat traps can be used either on screen frames with legs or on turntables, as shown above in figure 3. The screen frames have the disadvantage of exposing certain treatments or traps to larger numbers of gnats than others. Some of the treatments are also positioned in an environment which causes greater gnat activity. Due to this uneven exposure of certain traps to varying environmental conditions and varying amounts of gnat activity, the variation in the catch of traps on screen frames is greater than that of the traps placed on turntables. The turntables minimize this variation in the catch by equalizing the environmental factors and the directional activity of gnats.

Information of the amount of variation of traps under both conditions was obtained by placing traps on two screen frames and a turntable. Twenty traps were arranged on a turntable and 10 traps were placed on each of the two screen frames set on each side of the turntable. The results of this experiment indicate the variation to be greater for traps located on screen frames (table 2). The relative variation (coefficient of variation) in traps set on screen frames was found to be more than three times that of traps set on a turntable. Also, the variation in catches of traps on a turntable with four treatments and five replicates was equal to that of traps set on a screen frame (having the smallest variation) with two treatments and 10 replicates of each treatment (table 3). The variation in catch of traps set on another screen was twice that of traps set on the turntable with four treatments and five replicates. As is obvious from the data in table 3, smaller mean differences can be detected by the turntable than by the stationary screens for the same number of treatments and replicates.

The data obtained from turntables, therefore, will have more uniformity and reproducibility than data obtained from traps on screen frames. Turntables are employed whenever possible. Screen frames are used only for preliminary screening when a turntable cannot be used readily.

Effect of Lighting Level on Collections in C.E.S. Eye-Gnat Traps.—Most of the bait traps used for collecting eye gnats have been designed with a dark bait chamber (Parman 1932a, 1932b; Burgess 1935; Dow and Hutson 1958; Tinkham 1949). Into this chamber, light was admitted by judicious placing of glass containers, glass chambers, screen cones, and funnels, or by permitting the gnats to enter through a slit or opening into a lighted chamber, thus utilizing the positive phototropic response of eye gnats.

In the development of the C.E.S. eye-gnat trap, various degrees of lighting were obtained by dipping the funnels in black paint to the desired level below the neck of the funnel. Various lighting levels were tested in the field, and the catch in both bait dish and collection vial was counted.

TABLE 2.—Variation in collection of eye gnats in C.E.S. gnat traps placed on stationary screen frames and on turntable.[a]

Location of trap	No. of traps or replicates	Eye-gnat collection		Coefficient of variation (%)
		Range	Avg./Trap	
Turntable[b]	20	123–249	159.6	10
Screen frame I	10	59–433	184.4	32
Screen frame II	10	36–126	86.6	19
Screen frame I and II	20	36–433	135.5	37

[a]One-half-ounce egg bait used per trap.

[b]One revolution every 4.5 minutes.

TABLE 3.—Mean differences of the transformed variable ($y=\sqrt{\text{count}}$) expected (at the 75% confidence level) to be statistically significant with the number of treatments and replications indicated for stationary screen frames and rotary turntable.[a]

Type of frame	No. of treatments	No. of replicates per treatment	Mean differences	Coefficient of variation (%)
Turntable	2	10	1.40	11
Turntable	4	5	2.64	21
Screen I	2	5	7.65	59
Screen I	2	10	4.96	38
Screen II	2	5	3.16	34
Screen II	2	10	2.05	22

[a]For additional information see table 2.

The catch in the bait dish increased as the lighting level increased, but the collection in the vial remained relatively uniform at 30 mm. or higher lighting levels (table 4). The total number of gnats entering the trap increased as the lighting level increased, but a greater portion of these drowned in the liquid bait used in this experiment. Only gnats in the collection vials were counted in routine screening programs. Counting large numbers of gnats from the bait dish is difficult and does not add much to the value of the data except in a few special cases.

TABLE 4.—Efficiency of C.E.S. eye-gnat trap with various levels of lighting in the funnel[a]

Lighting level (mm.)[b]	Average No. of gnats per		Total
	Bait dish	Vial	
	In Sunshine		
0	15	5	20
10	30	19	49
30	188	99	287
50	283	95	378
70	329	91	420
100[c]	410	107	517
	In Shade		
30	—	106	
50	—	153	
70	—	182	
100[c]	—	183	

[a]Each treatment replicated five times on turntable. One-half-ounce egg bait used per trap.

[b]Lighting level determined by measuring the distance of the clear portion of the funnel from the neck to the black paint.

[c]Clear funnel.

A paint cover on the traps causes many difficulties in washing. The paint cover also chips during transportation, leaving clear spots on some of the funnels and thus introducing an additional variable into the experimental procedure. Drying the painted funnels in the oven also caused some difficulty.

The data presented in table 4 established the futility of darkening any portion of the C.E.S. trap; clear funnels were therefore employed in subsequent tests.

Bait Dish.—Various types of bait dishes can be employed as bait containers for the C.E.S. eye-gnat trap. The size of these dishes is of course limited by the size of the funnel used as a part of the C.E.S. eye-gnat trap. The funnels used here are 100-mm. Pyrex glass, and the diameter of a dish used as a bait container must therefore be smaller than 100 mm. When the dish is set on the screen support, there should be ample space between the sides of the dish and the sides of the funnel to permit unobstructed movement and flight of the eye gnats upward in the funnel.

Comparison of four types of glass dishes and one type of aluminum-foil dish demonstrated the importance of the type of bait containers as related to catch of eye gnats and *Milichiella* (table 5). Syracuse watch glasses and aluminum-foil dishes were more efficient than other containers, and resulted in a significantly larger number of gnats and *Milichiella* trapped than the traps with the other dishes. The catch with the staining dish was higher than that with the stender dish, and both of these were markedly lower than that with Syracuse watch glasses, and aluminum-foil dishes. The catch of eye gnats in traps with shell vial was significantly lower than that in traps with the other types of bait containers.

The influence of bait dishes on the collection of eye gnats in the C.E.S. eye-gnat traps can be correlated with the depth and surface area of the containers used. It is obvious from the data presented in table 5 that Syracuse watch-glass dishes and aluminum-foil dishes both have much greater surface area than the other dishes. They are slightly deeper than the staining and stender dishes, but much more shallow than the shell vial.

Aluminum-foil dishes were chosen because they

TABLE 5.—Effect of type of bait dish on efficiency of C.E.S. eye-gnat traps.[a]

Type of bait dish	Dimensions, mm.[b]	Capacity, fluid oz.	Surface area, cm^2	No. of gnats/trap		No. *Milichiella* per trap	
				Range[c]	Av.[c]	Range	Adjusted Av.[d]
Syracuse watch glass	52 x 13	0.75	21.22	113–236	168	4–11	7.6**
Aluminum-foil dish	57 x 18	1.75	24.61	45–240	164	1–17	10.5**
Staining dish	30 x 12	0.25	7.06	71–134	97	0–2	1.4
Stender dish	30 x 13	0.25	7.06	47–103	63	0–2	1.8
Shell vial	25 x 27	0.50	4.91	16–46	26	0–2	1.6

[a]The experiment was run for 4 hours. One-fourth ounce 0.2% egg bait (aged for 5 days) was used per dish.
[b]Diameter x depth (mm.)
[c]Eight replicates in randomized block design with four replicates on each of two turntables. Data transformed to $y=\sqrt{x}$ and analysis of variance completed. Any two means adjacent to the same line are not significantly different at the 0.01 level. The two turntables were significantly different from each other at 0.001 level. No significant difference was detected for the interaction between turntables and treatments.
[d]Eight replicates. Data transformed to $y=\sqrt{x+1}$ and analysis of variance completed. No significant difference was detected for the interaction between turntables and treatments.
**Significantly different from other treatments at the 0.01 level.

are inexpensive and can be discarded after use, thus eliminating the necessity of cleaning and washing the bait dishes after termination of each experiment. However, aluminum dishes cannot be used for strongly alkaline or strongly acidic liquid samples since aluminum undergoes a chemical reaction when in contact with such solutions. Paper portion cups have been found to be satisfactory for this purpose.

TABLE 6.—Escape of *Hippelates collusor* from untreated collection vials of the C.E.S. eye-gnat trap[a]

Trap No.	No. of gnats added	No. of gnats present and percent escaped after			
		2 hours		4 hours	
		No. present	Percent escaped	No. present	Percent escaped
1	30	17	43	16	47
2	28	16	50	12	57
3	25	15	40	10	60
4	24	19	21	13	46
5	25	13	48	11	56
6	23	19	17	13	44
7	24	18	25	11	54
8	23	19	17	13	44
9	24	18	25	12	50
Av.	25	17	32	12	51

[a]The traps with bait were arranged in the same manner in the laboratory as those in regular field screening tests.

Insecticide in the Collection Vial.—The presence of an insecticidal deposit in the collection vial is necessary for two reasons: 1) A quick knock-down insecticide immobilizes the insects in a short period of time in the collection vial, thus making it easier to count the catch at any time during and after the termination of an experiment. 2) The presence of a quick knock-down insecticide in the collection vial will immobilize and keep most of the captured insects from escaping back through the stem of the funnel. It has been observed that gnats readily escape back into the chamber of the trap. This was further substantiated by confining gnats in the collection vial in the laboratory and determining the rate of escape through the stem of the funnel over periods of 2 hours and 4 hours (table 6). At the end of 2- and 4-hour periods, an average of 32% and 51% of the gnats, respectively, escaped back into the bait chamber of the trap. In the presence of a quick knock-down insecticide, escape is greatly reduced.

An insectide that would increase the catch of the C.E.S. eye gnat traps should have low repellency and manifest quick-action potential against the test insects. Various organophosphorus insecticidal deposits were tried in the laboratory to determine the quick-acting toxicants against the gnats. One milliliter of petroleum ether solution of each material was placed in a 25-ml. Erlenmeyer flask and evaporated to dryness. The speed of action of the various toxicants used in the test is shown in table 7. Among the insecticides tested, Dibrom® proved to be the one having the quickest action, followed by dicapthon, malathion, and parathion, in that order. The remaining insecticides were relatively slow in action.

In order to determine the efficiency of organophosphorus insecticidal deposits, various materials were tested in a field experiment. One milliliter of 0.02% solution of each toxicant in acetone was added to each collection vial. The acetone solution was evaporated to dryness and the materials were subjected to testing 2 hours later. The duration of the test was deliberately lengthened

TABLE 7.—Speed of action of some organophosphorus insecticides against the eye gnat *Hippelates collusor*.[a]

Insecticide	% Conc.	Av. percent knockdown of gnats after						
		5 min.	10 min.	15 min.	20 min.	30 min.	40 min.	50 min.
Bayer 29493	0.01	0	0	0	8	78	98	100
	0.1	0	10	62	100	—	—	—
Dicapthon	0.001	0	0	64	98	100	—	—
	0.01	0	12	96	100	—	—	—
	0.1	0	60	100	—	—	—	—
Dibrom	0.0001	0	0	0	24	58	86	100
	0.001	100	—	—	—	—	—	—
Dylox	0.01	0	0	0	0	2	90	100
	0.1	2	8	18	38	86	98	100
Phostex	0.1	2	38	82	96	100	—	—
Korlan	0.001	0	0	0	4	98	100	—
	0.01	0	0	54	98	100	—	—
	0.1	4	48	100	—	—	—	—
Parathion	0.001	0	0	0	4	90	100	—
	0.01	0	2	16	82	100	—	—
	0.1	2	16	98	100	—	—	—
Malathion	0.001	0	0	0	0	68	98	100
	0.01	0	6	16	68	100	—	—
	0.1	2	44	92	100	—	—	—

[a]One-milliliter solution of each insecticide in petroleum ether was placed in a 25-ml. Erlenmeyer flask and evaporated to dryness. Twenty-five 3-day-old gants were placed in each flask. There were two replicates per treatment.

to accentuate the difference in count caused by the quick knock-down and repellency properties of the insecticides and by the escape of captured insects in the untreated control.

The presence of certain toxicants in the collection vial—for example, malathion, parathion, dicapthon, Bayer 22408, Dylox® and Korlan®—caused heavier catches of both *Hippelates* eye gnats and *Milichiella* species, as compared to the check treatment (table 8). Dibrom and DDVP, though quick knock-down materials, had a low catch of eye gnats in the vials. It is possible that these two materials exerted considerable repellency against the gnats. The efficiency of these materials influenced eye-gnat and *Milichiella* collection in the traps (see table 8).

Dicapthon deposits have been utilized in routine tests of attractants against eye gnats and *Milichiella* up to the present moment. A shift to the use of malathion in regular screening programs is considered for the future, malathion being easily available and safer than dicapthon, as far as mammalian toxicity is concerned. It should be pointed out, however, that the insecticidal deposits of one test should be eliminated before the vials are used in another experiment. In this way, the possibility of insecticide accumulation in the vials can be greatly diminished.

Influence of Type of Screen Support on Gnat Collection.—Two experiments were conducted on the role of type of screen support on collection of eye gnats in the C.E.S. eye-gnat trap. Disks of standard window screen (16-mesh) and hardware cloth (8-mesh) were stapled to the underside of the turntable for trap supports. These disks were fastened alternately to the table, and there were 10 disk supports of each kind of screen on the turntable.

The experiments were run under environmental conditions of two kinds. Experiment A (table 9) was run in open shade, with a slight amount of direct sunlight available to a portion of the turntable at all times. Experiment B was run under such conditions that most of the turntable was in direct sunlight, with only a few of the traps located in open shade at any single period.

Under intense lighting conditions a larger number of gnats move upward into the vial rather than downward into the bait dish (table 9). In open shade or under moderate lighting conditions this trend is reversed. Because of strong positive phototropic response of eye gnats to daylight, this response of gnats in the trap is to be expected. With the availability of more light in the upper portion of the trap, the gnats manifest more activity and are attracted toward the direction of the light into the collection vial.

In both experiments (table 9, A and B), the ratio of gnat collection in the vial to the number of gnats drowned in the bait dish was greater for the 16-mesh screen than for the 8-mesh hardware cloth. This indicates that a greater portion of the total number of gnats entering the trap through the 16-mesh screen supports move into

the vial under both moderate and intense lighting conditions. The passage of gnats back and forth through the 8-mesh hardware cloth is considerably easier than through the 16-mesh screen. It is possible that some gnats after entering the trap through the 8-mesh disk escape out of the trap.

TABLE 8.—Influence of insecticidal deposits in the collection vial on the catch of *Hippelates* eye gnats and *Milichiella* spp. in C.E.S. eye-gnat traps.[a]

Insecticide	Av. No. insects per vial[b]	
	Hippelates	*Milichiella*
Malathion	81 bc[c]	136 b[c]
Parathion	73 bc	130 b
Dicapthon	83 c	137 b
Bayer 22408	68 abc	95 ab
Dimethoate	59 abc	89 ab
Dylox	86 c	126 b
Korlan	84 c	121 b
Dibrom	41 a	120 b
DDVP	59 abc	101 ab
Control (Acetone only)	46 ab	69 a

[a]One milliliter of 0.02 percent solution of each insecticide in acetone was placed in each vial and evaporated to dryness. One-fourth ounce of 0.5 percent egg bait was used per bait dish. The experiment was run from 2:30 PM to 11:30 AM.

[b]Each treatment was replicated eight times with two replicates on each of four turntables. The treatments were arranged in randomized block design with two blocks on each of four turntables. Data transformed to $y=\sqrt{x}$ and analysis of variance completed. The interaction between treatments and turntables was not significant.

[c]Any treatments denoted by the same letters are not significantly different from each other at the 0.01 level.

Sixteen-mesh screen supports are preferred for attractant studies over 8-mesh or coarser-mesh hardware cloths for the following reasons:

1) The 16-mesh screen completely excludes house flies and other, larger insects that might be attracted to the materials evaluated. Small-sized flies can get through the hardware cloth and interfere with the attractancy of test materials.

2) The ratio of vial collection of gnats to bait-dish collection is higher for the 16-mesh screen support than for the hardware cloth.

3) The 16-mesh screen disks do not permit easy passage of gnats through it unless an attractant is placed in the trap. In an experiment in which traps with water alone were alternated with traps containing egg bait (10 replicates each) on 16-mesh screen disks, the average catch of eye gnats in traps with water was less than one gnat per trap as compared to an average of 100+ gnats per baited trap. In a similar experiment but using 8-mesh hardware-cloth disks, the average catch in the water treatment was 1.5 gnats per trap as compared to 37.8 gnats in traps baited with fermented egg.

Coarser-mesh disks such as those of 8-mesh or coarser-mesh hardware cloth have certain advantages in the initial screening of chemical samples. In the initial phases of investigations on the attraction of synthetic materials to eye gants, 8-mesh hardware-cloth disks are most desirable. By the use of these disks, information on a broader spectrum of attracted insects would be obtained. However, the limitation inherent in the use of these disks, as described previously, should be known prior to undertaking an attractant evaluation program.

TABLE 9.—Influence of type of screen support on collection of *Hippelates* eye gnats in the C.E.S. eye-gnat trap.[a]

Type of screen support	Av. No. gnats/trap			Ratio of vial to dish x 100
	Bait dish	Vial	Total	
	Experiment A[b]			
Window screen (16-mesh)	43	33	76	77
Hardware cloth (8-mesh)	43	28	70	67
Statistical significance[c]	N.S.	N.S.		
	Experiment B			
Window screen (16-mesh)	26	51	77	200
Hardware cloth (8-mesh)	44	62	108	141
Statistical significance[c]	+++[d]	N.S.		

[a]One-fourth ounce of 0.5 percent egg bait aged 5 days was used in each trap. Each treatment was replicated 10 times at random.

[b]Experiment conducted in open shade with one-fourth of the traps exposed to direct sunlight at all times. Gnat activity was lower as compared to that observed during the progress of experiment B.

[c]Data transformed to $y=\sqrt{x}$ and analyzed for analysis of variance.

[d]Significantly different from each other at 0.001 level.

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PROVISIONAL EMBRYONIC CUTICLES OF DYSDERCUS CINGULATUS (HEMIPTERA: PYRRHOCORIDAE)[1]

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ABSTRACT

In this insect two provisional embryonic cuticles are successively secreted before the definitive cuticle is produced. The first is incompletely formed, with only the cuticulin layer present. During each molt of a provisional cuticle the epidermal cells show definite signs of increased activity. Shedding of a provisional cuticle seems to be induced in order to synchronize the rate of cuticle deposition with the development of muscle ends, so that proper integration can take place between the muscle and the cuticular exoskeleton. Also, shedding of such a cuticle permits the epidermis to make necessary adjustments so as to act as a suitable mold for developing organs and tissues. Previously ascribed roles of provisional cuticles, such as protection after hatching or functioning as egg bursters, are secondarily acquired and may be described as preadaptations. The presence of neurosecretory cells of the brain, of a pair of thoracic glands, and of corpora allata in embryos of this species is confirmed; significantly, the thoracic glands are well formed and active when the provisional cuticles are molted.

The presence of a provisional embryonic cuticle has previously been reported in several insects, such as Aphidae, Orthoptera, Hemiptera, Neuroptera, and some types of Coleoptera (Wigglesworth 1950). A cuticular sheath of this kind also occurs in some spiders and crustaceans, suggesting that its production is an arthropodan feature.

This provisional embryonic cuticle, however, should not be confused with the serosal cuticles which have been described in several insects by Slifer (1937) and others.

Various roles have been ascribed to it. It has been thought to act as an "egg burster" (Sikes and Wigglesworth 1931) at the time of emergence in some insects. A protective role has also been ascribed to it on the basis of its assuming a protective function in some grasshoppers. For instance, the grasshopper emerges from the egg invested with a cuticular envelope which affords protection as the young nymph climbs upward through the soil from the buried egg pod to the surface. Though previous workers have emphasized the role of an "egg burster" or that of protection, no explanation has been put forward to account for the production of such an extra cuticle, particularly when it is shed before the insect emerges from the egg.

Sharan (1958, 1959) has pointed out that the main function of the provisional embryonic cuticle of *Locustana pardalina* (Orthoptera) and of *Tenebrio molitor* (Coleoptera) is to facilitate the attachment of the muscle bands with the second embryonic cuticle, and that the other functional interpretation is in the nature of secondary adaptation. The chief aim of the present work was to test the validity of conclusions reached in the cases of *Locustana* and *Tenebrio*. An attempt has also been made to describe briefly the tripartite endocrine glands of the insect during embryogenesis, and to determine whether they exert any influence on the process of molting the provisional cuticles.

The study of the provisional embryonic cuticles of *Dysdercus cingulatus* proved all the more interesting because, in this insect, two such cuticles were noticed instead of the usual one. Histochemical tests on the first embryonic cuticle revealed that it was an incomplete one, in the sense that only the cuticulin[2] layer of the epicuticle[2] was completely formed.

MATERIALS AND METHODS

Adult females of *Dysdercus cingulatus* (Fabr.) were reared in the laboratory. Eggs were collected and kept at a constant temperature of 30° C. with 100% relative humidity. Under these conditions almost all the nymphs hatched out in about 120 hours after the eggs were laid. Young embryos were dissected out from eggs at regular intervals, and as much yolk as possible was removed from them before fixation. Proper fixation of internal soft tissues of arthropods with hardened exoskeletons has always presented difficulties which, in the present study, were overcome by using hot alcoholic Bouin's fluid and leaving the material in the fixative for 6 to 12 hours at 48° C. Absolute alcohol was avoided during the final stages of dehydration, and clearing was done in amyl acetate. Paraffin melting at 56° C. was used for embedding, and serial sections were then prepared of embryos at different stages of development. Of the several stains tried, Mayer's haemalum and alcoholic eosin gave the

[1]Sponsored by A. Glenn Richards. Accepted for publication February 1, 1960.

[2]The terms "cuticulin" and "epicuticle" are used in the sense of Wigglesworth (1947). Lower (1959), in a paper that came to our attention after the present work was done, has proposed that the term "cuticulin" be discarded, that "epicuticle" be restricted to the "cuticulin layers" of Wigglesworth, and that a new, inclusive term "amphion" be used for the outer (polyphenol, wax, and cement) layers that were included in the "epicuticle" of Wigglesworth and other authors.

best results. The ammoniacal silver nitrate test of Lison (1936), as modified by Wigglesworth (1948), was used for detecting the first sign of deposition of the provisional embryonic cuticle.

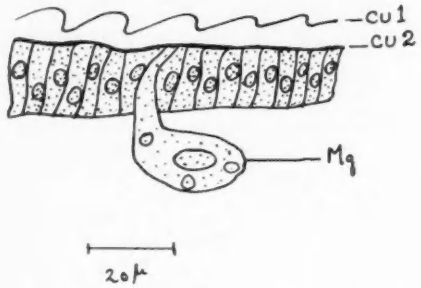

FIG. 1.—Portion of the epidermis at the 89-hour stage. Cu_1, first provisional cuticle; Cu_2, second provisional cuticle; Mg, dermal gland.

For determining the presence of different layers in the epicuticle, the method followed was mainly that devised by Wigglesworth (1948). The different processes, in succession, were: (a) dissecting out the embryos at the 85-hour stage; (b) treating the embryos for 1 hour in cold chloroform; (c) boiling the embryos in chloroform for 1 hour; and (d) immersing the embryos treated under (b) and (c) in ammoniacal silver nitrate solution. The argentaffin reaction indicates the presence of polyphenol in the cuticle. Determination of the presence of cement or wax in the outermost layer depends on the facts that cold chloroform readily dissolves the wax layer but not the cement layer, while hot chloroform dissolves both cement and wax layers. As a control test, one of the embryos was put in ammoniacal silver nitrate without first being pretreated with chloroform.

OBSERVATIONS

The Embryonic Cuticle.—The provisional embryonic cuticle was first detected in the 60-hour stage, and was shed at the 89-hour stage. A second provisional cuticle was soon deposited in its place, and this cuticle separated from the epidermis at the 102-hour stage (fig. 2). A third cuticle was soon deposited (fig. 3), which may be termed a definitive cuticle. To it the muscles were properly attached, and it formed the exoskeleton of the newly emerged nymph (fig. 3).

None of the provisional cuticles of *Dysdercus* showed any sign of exocuticle on histological examination, though the epicuticle was discernible as a very thin, black line in sections. Histochemical tests, which could be performed only on the first provisional embryonic cuticle, showed complete absence of both cement and wax layers. The polyphenol layer was not spread uniformly over the cuticulin layer, as the embryos treated directly with ammoniacal silver nitrate showed silver precipitated in a polygonal pattern, and the same pattern was obtained on embryos treated with cold and with boiling chloroform, respectively. No direct test could be performed for the cuticulin layer, but its presence is indirectly confirmed by the fact that the secretion of the molting glands readily, and almost wholly, dissolves old endocuticle but has no action whatever on the cuticulin layer of the epicuticle (Wigglesworth 1950). This layer remains present throughout embryogenesis.

The Epidermis.—At the time of deposition of the first provisional cuticle, the tissues were in early stages of differentiation and almost all the cells, including the epidermal ones, were intensely active. The epidermis was examined particularly with a view to detecting any cyclical changes effected in it coinciding with deposition and molting of the embryonic cuticles.

Throughout the early stages of embryogenesis the epidermis was found to remain very active, and no special changes were detected during the time when the first provisional cuticle was secreted. At the 89-hour stage (i.e., the time when retraction of the epidermis from the first provisional cuticle took place) the epidermis continued to be active. Its cells had become elongated, and some unicellular glands appeared adjoining the epidermal cells. Each gland was much larger than other epidermal cells, and was provided with a duct which opened toward the

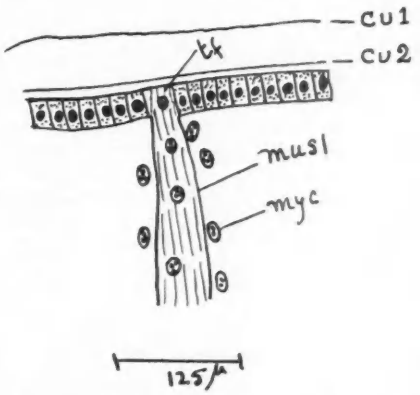

FIG. 2.—Condition of the epidermis, muscles, and cuticles at the 102-hour stage. Cu_1 and Cu_2, first and second provisional cuticles, respectively; musl, unstriated muscle fibers; myc, myoblast; tf, tonofibrillae.

outer side of those cells (fig. 1). As these glands appeared and were active only at the time of molting the provisional cuticles, it was presumed that they were the dermal glands, whose chief function is to secrete the cement layer of the epicuticle (Wigglesworth 1947). The dermal

glands were formerly regarded as the source of molting hormone (Verson 1911), but von Buddenbrock (1930) showed that they could not possibly be concerned with the molting process as their secretion was not discharged until later.

The epidermis was active again, with elongate cells, and the same type of active dermal glands were present, when the second provisional cuticle separated from the epidermis.

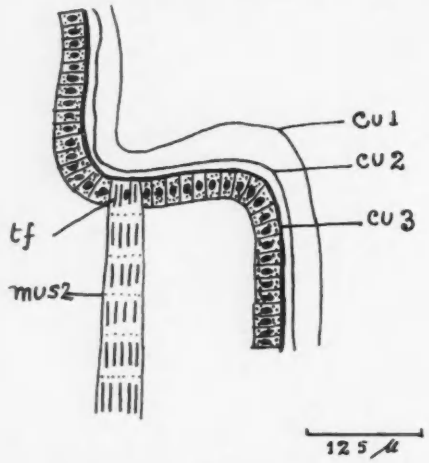

FIG. 3.—Condition of the epidermis, muscles, and cuticles at the 110-hour stage. Cu_3, definitive cuticle; mus2, striated muscle fibers. Other abbreviations as in figure 2.

Muscles.—About 3 days (70 hours) after the laying of the eggs, the first anlagen of the muscles were located in thoracic pouches, in limbs, and in abdominal pouches, in the form of a large number of myoblasts surrounding definitive muscle tissues. As might be expected of skeletal muscles, with which the present investigation was principally concerned, most of the muscles were found to arise in close connection with the epidermis. As development progressed, additional ones were formed, and at about the 80-hour stage muscles made their appearance in the head region. At this time the muscles were elongated and somewhat in formative stages; they took proper shape and were quite well formed at the 102-hour stage, but as yet were unstriated and showed myoblasts persisting between their fibers (fig. 2). The characteristic striated appearance developed at about the 110-hour stage, i.e., about 10 hours before emergence (fig. 3).

Mode of attachment of muscle fibers.—Muscle fibers are usually attached to the cuticle by intermediary fine, connective fibrils, the tonofibrillae, which traverse the epidermal layer. Tonofibrillae are produced by a transformation of the epidermal cells at the end of the muscles into cuticular fibrils that are continuous with the cuticle. In *Dysdercus*, when the tonofibrillae were forming in the plasmatic part of the cells, nuclei might entirely disappear from that part of the epidermis. Further observation on attachment of muscle fibers to the cuticle showed that where the tonofibrillae were formed, the nuclei of the epidermis persisted in between the groups of tonofibrillae (fig. 3).

In many insects the tonofibrillae not only traverse the epidermal layer but also penetrate the cuticle for some distance. Henneguy (1906) explained this by pointing out that the tonifibrillae are formed in the early stages when the epicuticula are being secreted, so that they are first connected with the outer layer of the exocuticula, then later, when the endocuticula is laid down, have their proximal extensions embedded in it. No such penetration of the cuticle by tonofibrillae was observed in *Dysdercus*.

ENDOCRINE GLANDS

The tripartite endocrine system and its composite hormonal mechanism have attracted the attention of several workers, most of whom, however, were chiefly concerned with roles of hormones during postembryonic stages, especially during metamorphosis. Thus far only Jones (1956) has described the tripartite endocrine glands in insects during embryogenesis. In the present investigation neurosecretory cells, thoracic glands, and corpora allata were observed, and special attention was directed to determining whether these glands were concerned in the molting of the provisional embryonic cuticles.

Neurosecretory cells.—These cells in the brain of *Dysdercus* were observed at different stages. When they were at rest it was practically impossible to distinguish them from the ordinary nerve cells, but when maximally active they were enlarged to three or four times the size of the latter and their enlarged nuclei stained more intensely.

In sections through the protocerebrum the neurosecretory cells were distributed around the periphery and thus lay immediately beneath the outer membrane; their outlines were either oval, square, or pear-shaped. They reached their maximum size and histological activity at 82 hours, and remained in this condition until the 96-hour stage. They then slowly became smaller, and soon thereafter it became difficult to distinguish them from other cells of the brain.

Thoracic glands.—These glands, in *Dysdercus*, were stated by Wells (1954) to arise as a pair of ectodermal invaginations into the second maxillary segment and, thereafter, to grow backward in company with the salivary glands. Serial sections of the thoracic glands were examined closely to determine their period of maximum activity, which is characterized by the presence of large nuclei with deeply staining secretory materials. The glands reached their peak period

during embryogenesis at about 89 hours, and their period of intense activity was observed until about the 100-hour stage. It covered, roughly, the time of molting of both the provisional embryonic cuticles.

Corpora allata.—These possessed the usual histological structure. It is, perhaps, valid to describe the appearance as syncytial, as no cell boundaries were visible at any stage during the growth of the embryo. The bean-shaped nuclei varied in size, and contained deeply staining spheres of chromatic material. Corpora allata did not show any cyclic changes during embryogenesis. They were properly formed and occupied their normal position on each side of the esophagus only at about 110 hours after laying of the eggs.

DISCUSSION

Although provisional cuticles are of common occurrence, their true significance has not previously been detected. Thus far, attention has been focussed on their functional value, as protective envelopes or as egg bursters. The functional aspects certainly are valid, but there is every reason to believe that these are secondarily acquired adaptations or preadaptations, as is commonly true of these kinds of adaptive structures.

The present work on *Dysdercus* has shown that the production of provisional embryonic cuticles is due also to developmental causes. They are produced during embryogenesis as the result of molts which are initiated to bring the rate of cuticle deposition into relation with the development of muscle ends; in fact, their production synchronizes these processes. The first and second provisional cuticles are laid down at times (60 to 89 hours, respectively) when the muscle ends are in an early stage of development. They are shed (at 89 and 102 hours, respectively) so that the muscle ends may gain proper attachment with the definitive cuticle. This interpretation is in complete agreement with the conclusions reached in the cases of *Locustana pardalina* and *Tenebrio molitor* (Sharan 1958, 1959).

It is worth considering also that the provisional embryonic cuticles (especially the first one, at 60 hours) are secreted at a time when the different organs and tissues are in early stages of differentiation. Therefore the epidermis, which acts as a mold for the developing organs and tissues, could not be in a position to perform its function unless it were free from the rigid exoskeleton for some time during embryogenesis. Shedding of the first, then the second, provisional cuticles provides the opportunity for the epidermis to make the adjustments in its outer form that are necessary for it to act as a suitable mold for the developing organs and tissues.

While it was possible to detect changes in the epidermis that corresponded to the shedding of the cuticle, we felt that more light could be thrown on production and molting of the provisional cuticles by attempting to detect changes in the endocrine glands. The relationship between the neurosecretory cells and the thoracic glands was resolved by Williams (1952), who found that a brain factor liberated by the former activates the latter to secrete their contributive hormone. The hormone of the corpora allata, according to Wigglesworth (1948, 1950), prevents the growth and differentiation in a larva or nymph towards the adult.

Examination of our serial sections of embryos of *Dysdercus cingulatus* made it clear that the molts of provisional cuticles during embryogenesis were accompanied by significant changes in different glands, as described above. The period of maximal activity of the neurosecretory cells was followed by that of the thoracic glands, and the latter coincided with the actual molt of the provisional cuticle. On the other hand, the corpora allata seemed to play no significant role in these moltings, and indeed they were themselves fully formed only after molting of the provisional cuticles had taken place.

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ROPALOPHORUS HALIDAY—A GENUS NEW TO NORTH AMERICA (HYMENOPTERA: BRACONIDAE: EUPHORINAE)[1]

ROY D. SHENEFELT[2]

ABSTRACT

The genus *Ropalophorus* of Haliday apparently has not been recovered since a single specimen was collected in 1834. Numerous specimens of a species closely related to the type species, *Microcotonus clavicornis* Wesmael, were collected in central Wisconsin. The type of the genus is discussed and the new species, *Ropalophorus wisconsinensis* is described.

The euphorine genus *Ropalophorus* was established by Haliday in Westwood's Synopsis (1840) for *Microctonus clavicornis* Wesmael. The generic name was quickly emended to *Rhopalophorus* by Blanchard (1840). This emended name was accepted by Brullé (1846), by Reinhard (1862), and by Provancher (1880, 1883, and 1886) who erroneously placed a number of species belonging to other genera in it and thus incorrectly recorded the genus as occurring in Canada. Foerster (1862) proposed the name *Eustalocerus*, pointing out that *Rhopalophorus* was preoccupied in Coleoptera by the name *Rhopalophora* of Serville. This change was adopted by Marshall (1887 and 1891), Provancher (1888), and Muesebeck (1936). Hincks (1943) pointed out that Serville's name was *Rhopalophora* and that *Rhopalophorus* was not preoccupied. The original spelling of the generic name is accepted here even though it was evidently incorrectly formed.

The genus was founded upon a single specimen taken by Wesmael in an osier bed May 27, 1834, in Belgium, probably near Brussels. According to Marshall (1887), "A British specimen was known to Haliday, who created a new genus for its reception, but left no written record of the species, and this has not been met with since." Marshall also included a wing figure of "*Eustalocerus clavicornis* Wesm., from Van Vollenhoven's copy of Haliday's drawing." Morley (1908) stated that only two specimens were known "from Belgium and England." Since Haliday named Wesmael's species as the type of *Ropalophorus*, it is, in the writer's opinion, entirely possible that Haliday had seen Wesmael's specimen and made the drawing from it, i.e., that the 'British' locality may be erroneous. This is especially true in view of Haliday's habit of keeping careful notes regarding his collections. The writer has no definite knowledge of other specimens; however, Muesebeck (1936) wrote, "*Eustalocerus clavicornis* has been recorded by several European authors as a parasite of *Ips typographus* (L.)." In any case, specimens of *clavicornis* must be regarded as being very uncommon. In view of the lack of known collections since 1834, the rediscovery of the genus in the north central portion of the United States is interesting.

During 1948 and 1949 light traps, bait traps, and yellow trap-boards (4″ x 9″ x ¼″ painted yellow and covered with cellophane coated with tanglefoot) were operated in the Nekoosa-Edwards Paper Company pulpwood storage yard at Nekoosa, Wisconsin, to trace the movement of insects within the yard. *Ips pini* Say was especially abundant. Among the insects captured were specimens which keyed to *Eustalocerus* but did not seem to agree fully with Wesmael's description of the first abdominal tergite ("Le premier segment est fortement élargi dans sa moitié postérieure, . . ."). Finally a specimen was sent, along with other material, to Mr. Muesebeck at the U. S. National Museum, who identified it as *Eustalocerus* and suggested that a note be written regarding the discovery of the genus in North America. However, the question of whether or not the specimens collected in Wisconsin were identical with *clavicornis* of Wesmael remained unanswered. Dr. A. Collart, chief of the Section d'Entomologie of the Institut Royal des Sciences Naturelles de Belgique, kindly loaned Wesmael's unique type for comparison. The descriptions which follow are based upon this specimen and upon a series of 93 specimens, all female, taken from the Nekoosa Yard. Drawings were made with the aid of a projector or by using squared grids.

Subfamily EUPHORINAE

Genus **Ropalophorus** Haliday

Ropalophorus Haliday 1840, IN: Westwood, Introd. Mod. Classif. Ins. 2, Synopsis: 61. Type: *Microctonus clavicornis* Wesmael, by monotypy and orig. desig.

Rhopalophorus, Blanchard 1840, Hist. Nat. Anim. articulés 3: 331.

Rhopalophorus, Brullé 1846, Hist. Nat. Ins. 4: 447.

Eustalocerus Foerster 1862, Verh. naturf. Ver. Preuss. Rheinland 19: 251. Type: *Microctonus clavicornis* Wesm., by monotypy and orig. desig.

Rhopalophorus, Reinhard 1862, Berlin. Ent. Zeitschr. 6: 327.

Eustalocerus, Marshall 1872, Cat. British Hymenopt. 113.

[1]Results of a cooperative project between the Wisconsin Agricultural Experiment Station, the Wisconsin Conservation Department and the Nekoosa-Edwards Paper Company. Accepted for publication January 18, 1960.

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Rhopalophorus, Provancher 1880, Naturaliste Canadien 12: 168 (misidentification).
Rhopalophorus, Provancher 1883, Petite Faune Ent. Canada 2: 515 (misidentification).
Rhopalophorus, Provancher 1886, Addit. Faune Ent. Canada, Hymenopt. 128 (misidentification).
Eustalocerus, Cresson 1887, Synopsis N. Amer. Hymenopt. 60.
Eustalocerus, Marshall 1887, Trans. Ent. Soc. London 1887: 67.
Eustalocerus, Provancher 1888, Suppl. Addit. Faune Ent. Canada Hymenopt. 378 (misidentification).
Eustalocerus, Marshall 1891, Spec. Hymenopt. Europe et Algérie 5: 22.
Eustalocerus, Dalla Torre 1898, Cat. Hymenopt. 4: 123.
Eustalocerus, Ashmead 1900, Proc. U. S. Natl. Mus. 23: 116.
Eustalocerus, Marshall 1901, Spec. Hymenopt. Eur. et Alg. 5 bis. Catalogue: 44.
Eustalocerus, Szépligeti 1904, Genera Insectorum fasc. 22: 173.
Eustalocerus, Morley 1908, Entomologist 41: 268.
Rhopalophorus, Lyle 1926, Entomologist 59: 262.
Eustalocerus, Muesebeck 1936, U. S. Dept. Agric. Misc. Publ. 241: 10.
Rhopalophorus, Hincks 1943, Entomologist 76: 101.

The genus *Ropalophorus* is readily separable from other euphorine genera by the short, geniculate, clavate, 10-segmented antennae which are inserted near the middle level of the eyes. The scape, first flagellar segment, and club are elongate, while the pedicel and segments 2 to 7 of the flagellum are subequal in width and length. The radial cell, measured along its anterior border, is subequal in length with the anterior margin of the stigma, and there are only two cubital cells. The median vein is not effaced towards its base, and the first abscissa of the cubitus is distinct.

Ropalophorus clavicornis (Wesmael)

Microctonus clavicornis, Wesmael 1835, Nouv. Mém. Acad. Sci. Bruxelles 9: 65.
Microcotonus clavicornis, Wesmael 1838, Nouv. Mém. Acad. Sci. Bruxelles 10: Plate, fig. M.
Ropalophorus clavicornis, Haliday 1840, *in*: Westwood, Introd. Mod. Classif. Ins. 2, Synopsis: 61.
Rhopalophorus clavicornis, Blanchard 1840, Hist. Nat. Anim. articulés 3: 331.
Eustalocerus clavicornis, Foerster 1862, Verh. naturf. Ver. Preuss. Rheinland 19: 251.
Rhopalocerus clavicornis, Reinhard 1862, Berlin. Ent. Zeitschr. 6: 327.
Eustalocerus clavicornis, Marshall 1872, Cat. British Hymenopt.: 113.
Eustalocerus clavicornis, Marshall 1887, Trans. Ent. Soc. London 1887: 68, Plate 5, fig. 3.
Eustalocerus clavicornis, Marshall 1891, Spec. Hymenopt. Europe et Alg. 5: 23, Plate 1, fig. 6.
Rhopalophorus clavicornis, Thomson 1895, Opuscula Entom. 20: 2166.
Eustalocerus clavicornis, Dalla Torre 1898, Cat. Hymenopt. 4: 123.
Eustalocerus clavicornis, Marshall 1901, Spec. Hymenopt. Europe et Alg. 5 bis. Catalogue: 44.
Eustalocerus clavicornis, Szépligeti 1904, Genera Insectorum fasc. 22: 173.
Eustalocerus clavicornis, Morley 1908, Entomologist 41: 287.
Rhopalophorus clavicornis, Lyle 1926, Entomologist 59: 262.
Eustalocerus clavicornis Muesebeck 1936, U. S. Dept. Agric. Misc. Publ. 241: 11.

The following notes, which are to be regarded as supplementary to Wesmael's excellent description of *clavicornis*, are based upon the type female, which is deposited in the Institut Royal des Sciences Naturelles de Belgique at Brussels. The pin bears the following labels: "Coll. Wesmael"; "***Mecroctonus*** [!] *clavicornis* mihi ♀ Det. C. Wesmael"; "Type"; and "Coll Wesmael Boite no 1". The specimen is in good condition, except that the club of the left antenna is missing and the thorax is split by the pin.

Length: 2.3 mm., excluding antennae and ovipositor. Length of abdomen one-fifth less than combined length of head and thorax. Ovipositor three-fifths as long as abdomen. Antenna subequal in length with thorax. Fore wing as long as body, its greatest width one-third the length. Hind wing three-fourths as long as fore wing, and one-fourth as wide as long.

Head transverse, twice as wide as long; occipital carina complete; face minutely punctate, with whitish pubescence; mesoscutum mostly smooth and shining, feebly punctate near notaulices; notaulices distinctly and sharply impressed and foveate but shallow, meeting before scutellar groove; scutellar groove divided into two large foveae by a central ridge; propodeum as long as first abdominal tergite from base to spiracles, rugulose, the posterior face sharply declivous, forming an angle of about 60° with the anterior face, emarginate medially and protuberant on each side of the emargination near the upper end; prepectus margined; mesopleuron shining, with a crenulate, curved sternaulus extending two-thirds of distance across base of mesepisternum; metapleuron roughened, reticulate-rugose like the propodeum. Legs slender, the posterior tarsus two-thirds the length of the posterior tibia; all tibial spurs short, not longer than width of tibiae at apex. First abscissa of cubitus complete; medius complete at base; radial cell as measured on wing margin shorter than length of stigma, not reaching wing apex. Stigma and venation pale brown. Abdomen petiolate, first tergite strongly bent at about the middle, broadening towards apex, with two large dorsal fossae before the spiracles, longitudinally aciculate behind spiracles; remaining tergites polished.

Color: chestnut-brown, excepting dark honey-yellow legs, maxillary and labial palpi, and most of mandibles. Apex of mandibles black, anterior (under) side of antennae paler than upper.

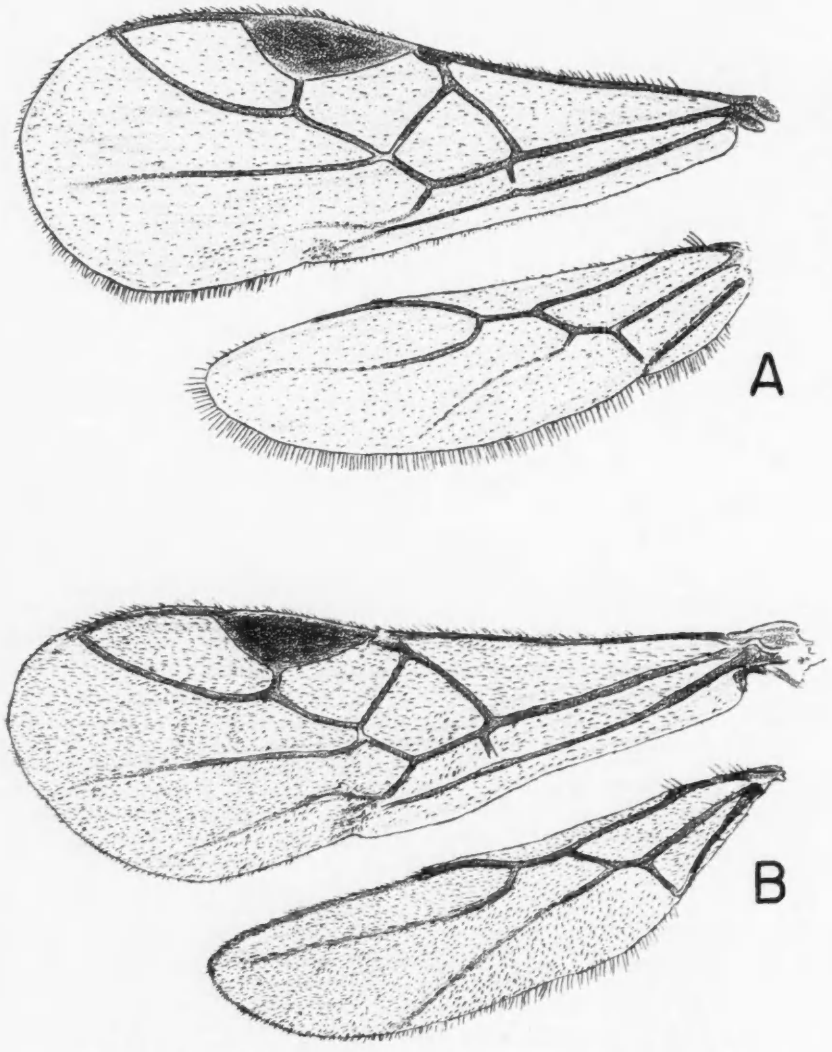
Ropalophorus wisconsinensis, new species

Differs from *clavicornis* in its larger size, greater relative width between posterior ocelli, more deeply impressed notaulices, narrower face, slightly longer radial cell, more robust legs and in many other minor characteristics.

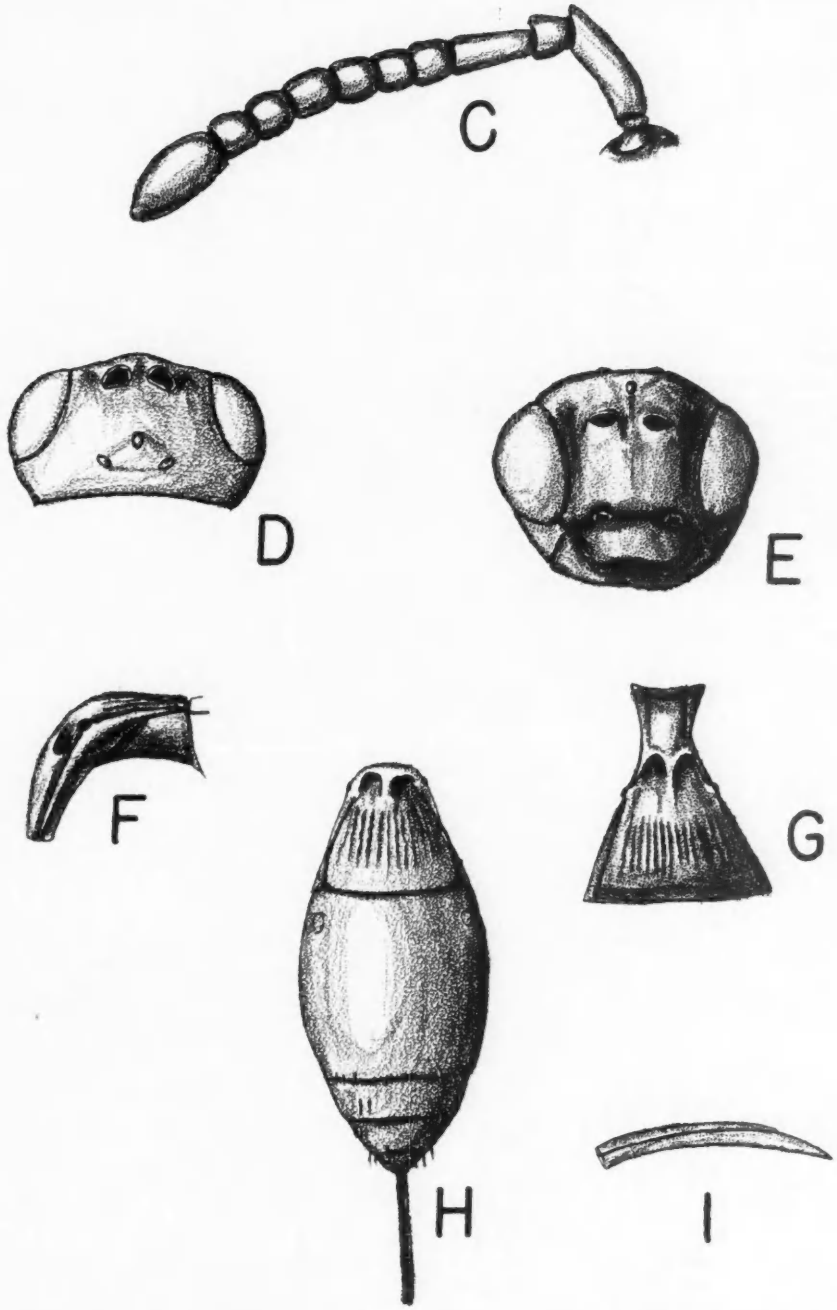
Holotype ♀—Nekoosa, Wood Co., Wisconsin. VIII–28–1948 R. D. Shenefelt. Light trap. In collection of University of Wisconsin.

Length: 2.6 mm., excluding antennae and ovipositor. Abdomen 1.06 times as long as combined length of head and thorax. Antenna 1.2 times as long as thorax. Fore wing 0.92 times as long as body, its greatest width 0.32 times its length. Ovipositor 0.92 times as long as abdomen.

Head: transverse, its greatest width 1.8 times its length; occipital carina complete; face minutely rugoso-punctate, with whitish pubescence; a short central ridge below the insertion of the antennae; relative width across bases of antennae 28, at narrowest point between eyes 22, the latter equal to distance from lower edge of antennal foveae to the deeply impressed groove between the anterior tentorial pits; clypeus doubly convex, with its apical portion reflexed and very shallowly emarginate; maxillary palpus five-segmented, the second segment enlarged, ovoid and as long as the first flagellar segment; labial palpi two-segmented, the second segment swollen, nearly securiform, with numerous setae on the flattened apex; mandibles bidentate, the teeth equal; antennae as in *clavicornis*, but with the segments possessing the following relative lengths commencing with the scape—20:7:11:6:6:6:5:6:5:17; ocell-ocular line 2.5 times the greatest diameter



A.—Left pair of wings of *Ropalophorus clavicornis* (Wesm.). B.—Left pair of wings of *R. wisconsinensis*, n. sp.



Ropalophorus clavicornis (Wesm.), type. C.—Right antenna. D.—Dorsal view of head. E.—Head, as seen perpendicular to face. F.—Left side of first abdominal tergite. G.—First tergite viewed perpendicularly to the spiracular area. H.—Abdomen viewed from above at right angles to second segment. I.—Lateral view of projecting portion of ovipositor.

of a posterior ocellus and equal to the distance between the posterior ocelli; eyes hairy, converging below; temple 0.67 times as wide as eye, smooth, with scattered, light-colored hairs; frons above antennae nearly flat, feebly coriaceous, with scattered hairs.

Thorax: Notaulices crenulate, narrow, sharply impressed to a depth approximately equal to their width, converging behind and forming a roughened, depressed area; mesoscutum smooth, excepting shallow pits at bases of hairs; prescutellar furrow deep, crossed at center by a single strong carina; scutellum smooth and rounded; dorsal face of propodeum rugose, the rugae forming numerous small areolae, the posterior edge broadly emarginate to a depth of one-third the length of the face; posterior face of propodeum deeply concave, with larger areolae than on the dorsal face, becoming smooth ventrally; sides of pronotum and central portion of mesopleura smooth; prepectal carina present; sternaulus and upper third of mesopleuron rugoso-punctate; metapleuron coarsely rugoso-areolate.

Legs: Relative lengths of portions of hind leg as follows: coxa 22 (width 15), trochanter 20, femur 47 (width 11), tibia 72, outer tibial spur 7 (subequal with inner), metatarsus 25, second tarsal segment 10, third tarsal segment 7, fourth tarsal segment 5, fifth tarsal segment 8, claws 5 (claws simple and weakly curved).

Wings: See figure B. In the fore wing the cubitus emerges from the recurrent nervure instead of from the first cubital cell as in *clavicornis*. The wing apex also is less blunt. In the hind wing, the radial cell is narrower than in *clavicornis* and the cubitus much longer and straighter.

Abdomen: Essentially as in *clavicornis*, but relatively longer; first segment not as strongly bent, the dorsal surface of the terminal portion forming an angle of approximately 60° with that of the anterior portion; the strongly compressed ovipositor longer than in *clavicornis*, more strongly decurved, more attenuated towards the tip and thus narrower and sharper, with a minute notch on dorsal side near the apex.

Color: Piceous except as follows: antennae brown, paler on anterior side; face brown; labrum, mandibles (except darkened apices), legs (excepting brown tip of last tarsal segment) yellowish; ovipositor yellowish with a brown streak along center on side; abdomen becoming paler towards apex and venter.

In the paratypes size ranges from 2.25 mm. to that of the holotype or very slightly larger; color of piceous areas in holotype is brown in some specimens. Structural features appear to remain remarkably constant except that the antennae vary about 10 percent in relative length. Specimens were collected in the Nekoosa-Edwards Paper Company pulpwood yard at Nekoosa from July 6, 1948, to September 7, 1948, and from June 8, 1949, to July 25, 1949.

Paratypes are to be deposited in the U. S. National Museum, the Canadian National Collection, the British Museum, the Institute Royal des Sciences Naturelles de Belgique, the Naturhistorische Museum in Wien, the Czechoslovak Academy of Sciences, and the Zoological Institute, Academy of Sciences at Leningrad.

BOOK REVIEW

AËDES AEGYPTI (L.), THE YELLOW FEVER MOSQUITO, ITS LIFE HISTORY, BIONOMICS AND STRUCTURE. By Sir Rickard Christophers. New York: Cambridge University Press. 739 pp. 1960.

Among mosquitoes, the subject of this volume is probably the single species that has received the most thorough study, and it is certainly one of the most important ones from the standpoint of public health and as a laboratory animal. Among specialists in the field of culicidology the author is fully as well known. Sir Rickard became a member of the Malaria Commission of the Royal Society and Colonial Office in 1899 and has devoted his life to the study of mosquitoes, particularly the anatomy of all stages, and the taxonomy of the Anophelinae. His broad knowledge and long experience makes a work of this sort particularly valuable. Very few species of insects have had so much information about them gathered together in one volume. While the work is principally a compilation taken from many widely scattered sources up through 1956, there is evidence of considerable original work, particularly on the anatomy of the brain and on embryology. Addenda (pp. 717–719) make the book more up to date.

As the title indicates, this work is devoted principally to the structure of the yellow fever mosquito in all its stages, and to its activities. There is a chapter devoted to the history of the study of mosquitoes in general and to *Aëdes aegypti* in particular, one on the systematic position and distribution, one on disease relationships and control, and one on the techniques of study and rearing. There are three chapters on the egg, including embryology and hatching, six on larvae, two on pupae, and 15 on the imago. These chapters cover the anatomy, both external and internal, physiology, behavior, and ecology, much of it in great detail. Each chapter is accompanied by its own, often extensive bibliography. There are 86 figures, most of them actually full page plates with a number of illustrations on each, and numerous tables of measurements and biological reactions.

There appear to be relatively few errors in the work. We do note that the type of *Culex fasciatus* is declared to be lost whereas it is in the Copenhagen Museum and that the first mention of the rotation of the male terminalia was by Christophers (1922) whereas it was actually Christophers (1915). This volume contains such a wealth of information not only about *Aëdes aegypti* but about mosquitoes in general that it should be on the shelf of anyone engaged in the study of mosquitoes, particularly in the laboratory.

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OBITUARY

GEORGE A. EDWARDS
1914–1960

Dr. George A. Edwards, Associate Research Scientist, in charge of the Laboratories for Micromorphology in the Division of Laboratories and Research, New York State Department of Health, Albany, died suddenly in Albany on March 1, 1960, at the age of 45. Dr. Edwards was born in Boston, December 12, 1914. He was a graduate of Tufts University, with specialization in biology and chemistry, 1939–1941; did graduate work in physiology and zoology at the Marine Biological Laboratory, Woods Hole, Massachusetts, and at the University of Pennsylvania, 1941–1943; and in 1947 received his Ph.D. in biology from Harvard University. Throughout this period Dr. Edwards was engaged in research in comparative anatomy and embryology. From 1947–1952 he was also Assistant Professor of Biology, Tufts University, where his research was concerned with fundamental problems in insect physiology. At the University of São Paulo, Brazil, 1952–1956, he was Professor of General Physiology, Coordinator of Research, and Co-founder of the Marine Biological Laboratory of the University.

In the Second World War, Dr. Edwards was Research Associate in the Office of Scientific Research Development, engaged in the development of micro-analytic technics for gas and fluid analyses. Following this he was attached to the Proving Ground Command of the U. S. Air Force with service in Alaska and the Aleutians in the development of equipment for high altitude flying and survival technics for air-sea rescue.

In 1956 Dr. Edwards joined the Research Laboratories of the New York State Department of Health, becoming also Associate in Anatomy at the Albany Medical College of Union University. In his more than 100 publications since 1942, Dr. Edwards presented his extensive research on nerve and muscle physiology, pharmacology, and morphology. His recent work in electron microscopy included studies of the micromorphology of fungi and bacteria, of human bronchogenic carcinomas, normal and transformed tissue culture cells, and on the sites and modes of virus formation in tumor cells, in particular, polyoma and leukemia viruses.

Dr. Edwards held membership in many professional and scientific societies, among them the Entomological Society of America, Cambridge Entomological Society, American Society of Zoologists, Gerontological Society, Inc., Biophysical Society, Electron Microscope Society of America, American Association for the Advancement of Science, Brazilian Society for the Advancement of Science, Corresponding Fellow of the Brazilian Academy of Sciences, New York Academy of Sciences, American Institute of Biological Sciences, American Association of University Professors, Sigma Xi.

Dr. Edwards had a rich and many-sided life. He was a student of philosophy; he had a deep interest in Brazilian history and culture; he was a musician of competence; and to his work in science he brought a superbly trained mind, an astounding intensity of application, and a selfless dedication.

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SCIENTIFIC NOTES

A New Character for the Identification of the Boll Weevil and the Thurberia Weevil (Coleoptera: Curculionidae)[1]

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The criteria given by Pierce (1913) for distinguishing the thurberia weevil (*Anthonomus grandis thurberiae* Pierce) from the boll weevil (*Anthonomus grandis* Boheman) have proven satisfactory when one confines his identifications to the populations that these criteria were meant to distinguish. One's identifications are doubly certain if he is dealing with the principal populations of the two. The boll weevil ranges all over the southeastern United States but is not known from west of Texas. The thurberia weevil is confined to Arizona in the United States. However, when one attempts to apply Pierce's criteria to the identification of unknown populations, he experiences some difficulty. Some boll weevils look very much like thurberia weevils and thurberia weevils sometimes look like boll weevils. Average differences may exist but it is not easy to compare samples on average differences alone. Any extension of the range of either weevil into new territory could have serious economic implications. If one has only a small sample for identification, it is easy to wish that someone else had to make the decision.

The taxonomic relationship of the two forms is still as obscure as it was when Pierce described var. *thurberiae*. Since his variety is geographic, it is now regarded as a subspecies. The known populations are allopatric and even this designation is subject to doubt. It is unlikely that much can be done toward an elucidation of the relationship between *grandis* and *thurberiae* without a study of the populations on the wild cottons of Mexico. The present paper is not directly concerned with the taxonomy but with the problem of identification of isolated populations in the United States as either *grandis* or *thurberiae* as they are now recognized. External structures have been examined by many workers. Therefore one would expect to have to turn to internal structures if he wanted to find some clear-cut difference that had not been noticed before. The author has done this and has found that the spermatheca of the female

[1]Arizona Agricultural Experiment Station Technical Paper No. 571. Accepted for publication February 15, 1960. Partial cost of publication of this paper was met by the author.

FIG. 1.—Spermathecae of *Anthonomus grandis* Boheman. Hope, Arkansas, June 10, 1954, Leigh and Elmore.

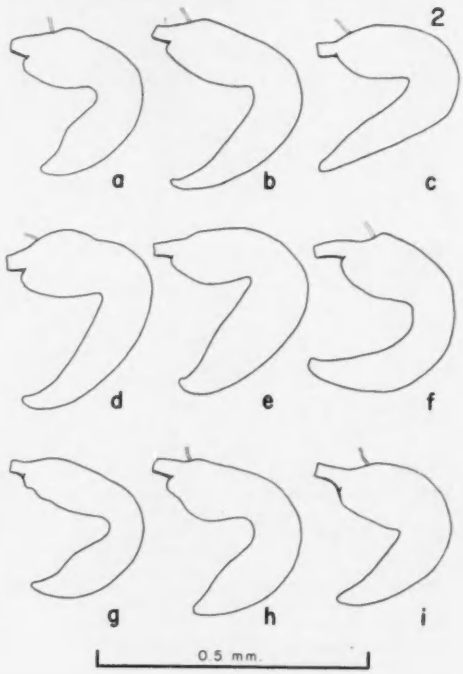

FIG. 2.—Spermathecae of *Anthonomus grandis thurberiae* Pierce. a-g. Carmen, Sta. Cruz Co., Arizona, Dec., 1955, W. Kauffman, on cotton. h. Molino Basin, Sta. Catalina Mts., Arizona, Aug. 22, 1955, G. D. Butler and F. G. Werner. i. Sta. Rita Mts., Arizona, Aug., 1937.

shows promise of providing a useful basis for identification.

The spermatheca is a small structure, barely 0.3 mm. across at its greatest dimension, tan in color and sclerotized. The walls are thin but are strong enough to withstand gentle handling. It is U-shaped and attached to a slender sperm duct near one end. A thin sheet of muscle extends from one arm of the U to the other; from its position it must serve to compress the spermatheca by bringing the two arms together. One arm is definitely closed at the end. The arm attached to the sperm duct is open and apparently connects with an accessory gland. A sclerotized tube forms this connection and it is this tube that is most different in the two weevils.

Figure 1 shows outline drawings of the spermathecae of nine females of the boll weevil from Hope, Arkansas. These were dissected from specimens preserved in alcohol, placed in glycerin mounts on microscope slides and drawn with the aid of a microprojector. Some specimens have the end of the sperm duct in place. The sclerotized tube is at the upper left of each drawing. It is from short to very short in all cases. A small sample from Roanoke, Texas falls well within the limits of variation shown here. Figure 2, a-g, shows the spermathecae of females from a sample of the thurberia weevil taken on cotton at Carmen, Arizona. This locality often has temporary infestations on cotton, presumably from wild cotton in the nearby Santa Rita Mountains. Figure 2, h and i, shows spermathecae of thurberia weevils taken on thurberia or wild cotton (*Gossypium thurberi*) in the Santa Rita and Santa Catalina Mountains. The sclerotized tube is much longer than in the boll weevil in all cases. Several other small samples from Arizona fall within the range of variation shown. The shape of the spermatheca itself seems to differ slightly in the two forms. The arm that is attached to the sperm duct and bears the sclerotized tube is relatively larger in most of the boll weevil specimens. However, the difference is small and the samples are not large enough for an evaluation. Perhaps it would be useful as an additional clue to the relationships of Mexican populations.

Despite the rather small size of the sample dissected, the size of the sclerotized tube of the spermatheca seems to offer a more useful character for distinguishing the boll weevil and the thurberia weevil in the United States than has been available in the past. Female specimens, at least, should be identifiable without reference to the locality label.

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Rearing Tyroglyphid Mites on Neurospora[1]

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In the course of preliminary investigations for a population dynamics study, we have successfully reared four species of tyroglyphid mites on *Neurospora*. Several authors (Solomon 1943, Hughes 1948, and Krantz 1955) have reported that a number of species of mites commonly found in stored grain and stored grain products probably feed on fungi and vegetable debris rather than on the grain itself. Rivard (1958a, 1958b) reported rearing *Tyrophagus castellanii* (Hirst.) on *Aspergillus* sp.

Mites were obtained from debris consisting of chaff, milled grain, and microflora, taken from the floor of a feed room on a local farm. The test populations set up from these collections were composed of at least four species, *Acarus siro* (L.), *Aleuroglyphus ovatus* Troup., *Caloglyphus berlesei* (Michael), and *Lardoglyphus* sp.[3] The mixed-species populations were housed in 100 x 15 mm. polysterene petri dishes. A hole approximately 2 inches in diameter was made in the top of the petri dish with a hot soldering iron. A ring of petrolatum-paraffin oil mixture was placed on the inside, around this hole, then the top was fastened to the bottom with dichloroethane. The vertical edge of the top was next removed, leaving a neat, flat, container which could easily be stored and in which the mites could easily be observed. These containers were kept in a 1-gallon jar, in which the relative humidity was kept at 73% by means of a saturated NaCl solution. Room temperature was held at 80°±2° F.

The mites were fed a melon mutant strain of the ascomycete *Neurospora crassa* Shear and Dodge.[4] The characteristics of this strain were described by Srb (1957). The culture medium was that described by Beadle and Tatum (1945), and was used both in 50-ml. Erlenmeyer flasks and in 13 x 100-mm. culture tubes. Every 3 days a weighed amount of *Neurospora* was introduced into the population cage on an aluminum foil plate. The mites consumed between 0.01 mg. and 0.05 mg. per mite per day.

The life cycle of one species, *Caloglyphus berlesei*, was examined. A female, reared on *Neurospora*, was isolated and her eggs were collected. These eggs hatched in 24 to 48 hours, and the progeny deposited eggs on the ninth day. Thus the life cycle, from egg to egg, was 9 days under the laboratory conditions.

[1]This work is being supported by National Science Foundation Grant G-8903. Accepted for publication December 19, 1959.

[2]Participant in the 1959 National Science Foundation Teacher Research Participation Program.

[3]The authors thank Dr. E. W. Baker, Entomology Research Division, U. S. Department of Agriculture, for identifying these mites.

[4]We are indebted to Dr. Adrian M. Srb, Department of Plant Breeding, Cornell University, for furnishing several mutant strains of *Neurospora*.

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A Simple Method for Sexing Nymphal German Cockroaches[1]

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In the course of certain investigations dealing with the German cockroach, *Blattella germanica* (L.), it became necessary to separate males and females prior to the final molt. To our knowledge, no method of sex determination in nymphs has previously been reported for this species. It is usually quite easy to distinguish the sexes in the last one or two nymphal instars by gross appearance, however, and this means has probably been used in the past. In the present paper a method is described by which it is possible to sex the nymphs in all instars including the first. The distinguishing characters are the presence or absence of a notch in the ninth sternum in the early instars and, in later instars, the

[1]This work was partially supported by a grant from the U. S. Public Health Service, and partial cost of publication was met by the Virginia Agricultural Experiment Station, Entomology Department. Accepted for publication February 26, 1960.

[2]The assistance of Dr. E. M. Raffensperger in studying the morphology of the cockroach is gratefully acknowledged.

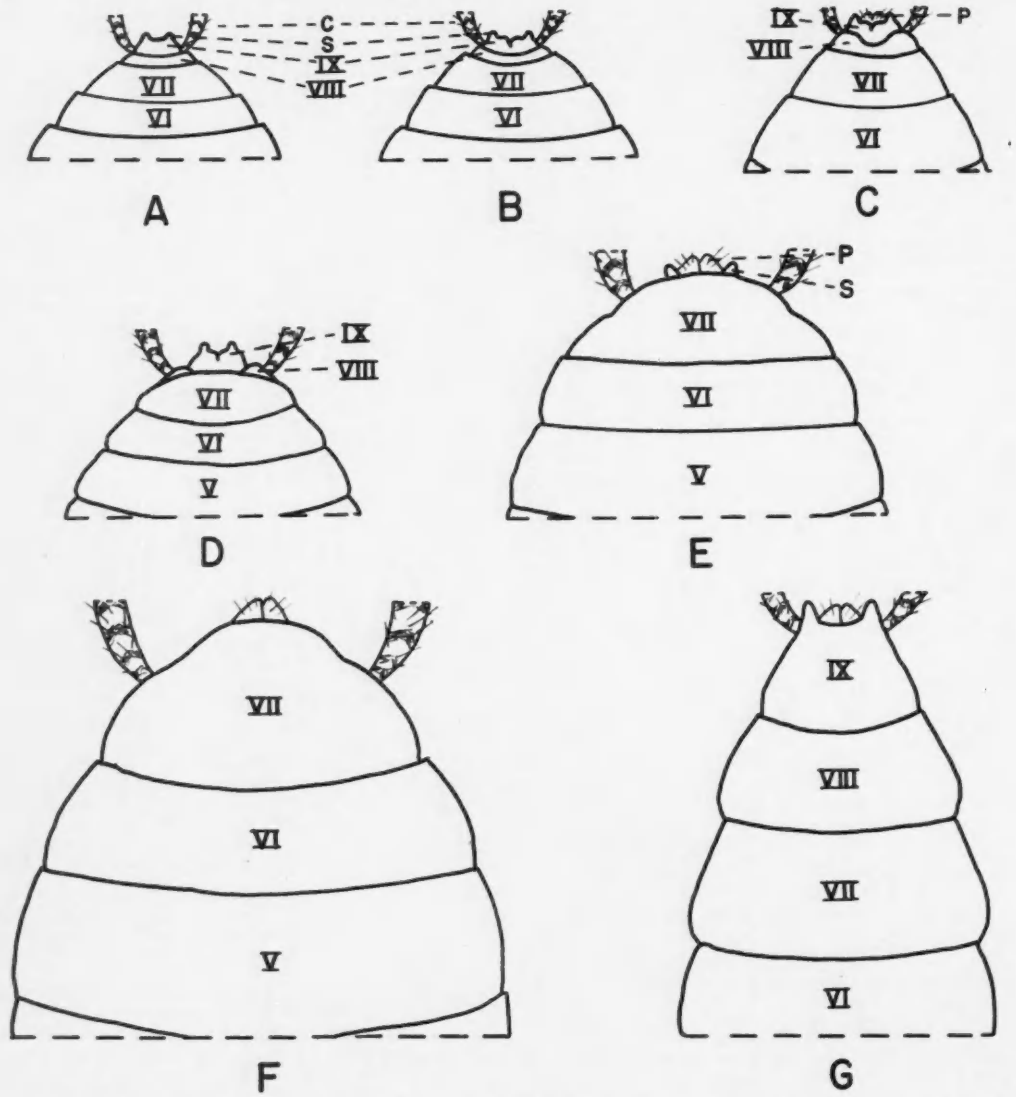

FIG. 1.—Ventral view of the terminal abdominal segments of nymphal German cockroaches. Sterna are numbered with roman numerals. C=cerus; P=paraproct; S=stylus. A, first instar male; B, first instar female; C, second instar female; D, third instar female; E, fourth instar female; F, sixth instar female; G, sixth instar male. A and B, 35×; C, 30×; D-G, 15×.

presence or absence of the eighth and ninth sterna. Over 800 nymphs have been sexed by this method without error.

A scale of reference was needed to facilitate description of changes that occur in different stages of nymphal development. Woodruff (1939) described several morphological characters, such as greatest width of head, which can be used for this purpose in the German cockroach. By the use of these characters he found six nymphal instars, but was unable to sex the nymphs by any of these traits. In the present paper another type of measurement was made which does show a difference due to sex. This parameter is body length from the top of the head to the end of the abdomen. Measurements of over 300 nymphs indicate that body length also falls roughly into six nymphal groups. In these groups the majority of lengths vary within a rather narrow range, as shown in table 1, with the ranges for females being higher in later instars. Considerable variation does occur outside these limits and is probably the result of a number of factors, among which is the ability of these insects to telescope their abdominal segments. In spite of this variation these groups were precise enough for the present work. Accordingly, they were

TABLE 1.—Groups of body lengths used in estimating the number of instars of *Blattella germanica*.

Instar	Body length (mm.)	
	Males	Females
1	2.8– 3.0	2.8– 3.0
2	4.4– 4.6	4.3– 4.7
3	5.2– 5.6	5.5– 6.0
4	6.6– 7.5	7.0– 8.2
5	8.5– 9.6	9.5–10.4
6	10.6–11.5	12.5–13.5

given instar numbers from one to six by which they will be referred to in the remainder of this paper (see table 1). It should be recognized, however, that this is not intended to be an absolute method for instar determination.

The designation of first-instar nymphs is unequivocal because here only newly hatched nymphs were used for purposes of measurement and description. In this stadium nine sterna are present in both males and females. The ninth, or terminal sternum, is relatively small and bears two minute styli. A median notch on the caudal margin of this sternum is present in females but not in males, as shown in figure 1, A and B. A similar notch was reported in female nymphs of *Periplaneta americana* (L.) (Gould and Deay 1938), although, in this case, its first appearance was in the second instar. Possibly this criterion will be useful for nymphal sex determination in other genera of Blattidae. It can be used to determine sex in all except the last instar of *B. germanica*.

With large nymphs it is necessary to use a different character to separate the sexes. Sternal number serves as a convenient tool. In male nymphs nine sterna are retained throughout their development. In all instars the males show little change in their sterna other than a progressive increase in size (fig. 1, A and G).

In females the situation is somewhat different. Second-instar females are quite similar to those of the first instar, except for a strong median indentation of the caudal margin of the eighth sternum (fig. 1, C). A weaker indentation is present in some, but not all, females of the first instar. In third-instar females, the ninth sternum is exposed but the central portion of the eighth is usually hidden by the overlying seventh sternum, causing the eighth to resemble lateral lobes (fig. 1, D). In some specimens the seventh completely covers the eighth sternum. In this instar the seventh, which becomes the subgenital plate in adult females, is noticeably larger than the other sterna.

In the fourth and fifth instars the eighth sternum is not present in female nymphs, and the ninth is completely hidden by the large subgenital plate, except for the styli (fig. 1, E). The ninth sternum, with its distinctive notch, can be exposed by pushing back the subgenital plate.

In the last instar the nymphs are very large, and the elongate, tapering abdomen of the males contrasts markedly with the broader, rounded abdomens of the females. Usually it is possible to determine sex in this and the preceding instar by gross appearance. In the last instar, however, females are readily recognized by the presence of only seven sterna (fig. 1, F), as opposed to nine in males. Also, in the last one or two instars females have 8 and males have 10 abdominal tergites.

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The Pupae of *Heliothis zea* and *Heliothis virescens* (Lepidoptera: Noctuidae)[1, 2]

H. H. NEUNZIG

The corn earworm, *Heliothis zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (Fabricius), have several host plants in common, including cotton and tobacco. At times, therefore, the eggs and larvae of both species are found on the same plant, and pupae of both species occur together in the soil.

It is not possible, with information currently available, to separate the ova, the early-instar larvae, and the pupae of the corn earworm from these same stages of the tobacco budworm. Studies on the comparative morphology of the immatures of these two species have heretofore resulted in the establishment of diagnostic characteristics which apply only to the later larval instars (Garman 1920, Crumb 1926, Brazzel et al. 1953). The present paper is devoted to making information available which would be of use in separating pupae of the corn earworm from pupae of the tobacco budworm.

METHODS AND MATERIALS

Pupae utilized for study were derived from insects reared in the laboratory. The laboratory cultures originated from last-instar larvae collected in the field in North Carolina, from a number of host plants including tobacco, cotton, and corn. In all, several hundred pupae of the corn earworm and an equal number of pupae of the tobacco budworm representing eight different populations were examined during the investigation. All pupae were fixed in K.A.A.D., and preserved in 95 percent alcohol to permit long-term study. Measurements of the length and the width of the pupae were taken prior to fixation and preservation.

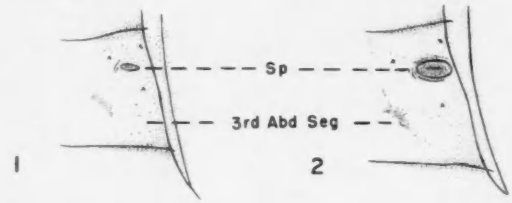
OBSERVATIONS

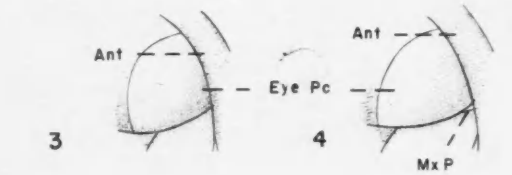
One of the most apparent and consistent structural differences between the pupae of the two species was found in the size of the abdominal spiracles in relation

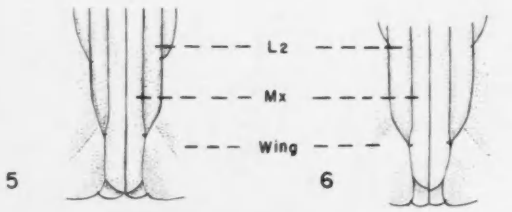
[1]Contribution from the Entomology Department, North Carolina Agricultural Experiment Station, Raleigh. Published with the approval of the Director of Research as Paper No. 1098 of the Journal Series. Accepted for publication November 30, 1959.

[2]The assistance of W. D. Duckworth is gratefully acknowledged.

to the size of the segments. These spiracles were rather large and wide in corn earworm pupae, while in tobacco budworm pupae they were relatively small and narrow. In the corn earworm, on segment 3, the ratio of the narrow dimension (or "width") of the spiracle to the length of the segment averaged 1:6.5 (range, 1:5.5 to 1:7.0). In the tobacco budworm this ratio averaged 1:14.6 (range, 1:12.0 to 1:16.0). Portions of the third abdominal segments of the two pupae are illustrated in figures 1 and 2.







FIGS. 1 (*Heliothis virescens*) and 2 (*H. zea*).—Lateral view of part of third abdominal segment and adjacent sclerites of pupa, showing spiracle (Sp).

FIGS. 3 (*H. virescens*) and 4 (*H. zea*).—Lateral view of part of pupal head, showing eye piece (Eye Pc), antenna (Ant), and maxillary palpus (MxP).

FIGS. 5 (*H. virescens*) and 6 (*H. zea*).—Ventral view of terminal portions of pupal maxillae (Mx), mesothoracic legs (L_2), metathoracic legs, antennae, and wings.

Pupae of the two species also differed in regard to possession of a pair of small, triangular sclerites, the maxillary palpi. These, when present, are located caudad of the eye pieces and along the cephalic margins of the prothorax (see figure 4). They were seen in 96.5 percent of the corn earworm pupae, but never in pupae of the tobacco budworm. In several of the corn earworm pupae examined only one maxillary palpus was apparent. Also, in a few instances, the forking of the suture which gives rise to the palpus occurred below the surface, resulting in the structure being visible but beneath the integument.

A third difference exhibited by pupae of these species concerned the extent of the contact existing between the terminal portions of several of the appendages (see figures 5 and 6). The maxillae and the wings usually were contiguous over a greater area in the tobacco budworm than in the corn earworm; and, conversely, the mesothoracic legs and the wings were in contact over a greater area in the corn earworm than in the tobacco budworm. This relationship can be expressed as follows. In tobacco budworm pupae the length of the contact area between the wings and the mesothoracic legs averaged 1.4 (range, 0.6 to 3.8) times greater than that between the wings and the maxillae; while in corn earworm pupae the former averaged 3.5 (range, 1.0 to 11.0) times greater than the latter. This feature was found to be somewhat variable, but the figures illustrate the relationships as they occurred in the majority of specimens.

The cremaster is of considerable importance in the separation of a number of the pupae of the Noctuidae, but this structure was found to be similar in the two species treated here. The enlarged basal portions of the pair of spines were somewhat more pronounced in the corn earworm, but the difference seemed too slight to be of value.

Mosher (1916) characterized pupae of the genus *Heliothis* (*Chloridea*) as possessing very fine punctations on the cephalic one-third of abdominal segments 5, 6, and 7. Pupae of both species examined in this study possessed punctations not only on these segments, but also on the cephalic one-third of abdominal segment 4. The punctations on segment 4, in both species, were considerably fewer and slightly more shallow than those on the more caudal segments.

The corn earworm pupae averaged 21.6 mm. (range, 17.0 to 26.0 mm.) in length, and 6.0 mm. (range, 5.0 to 7.2 mm) in width across the thorax. Tobacco budworm pupae averaged 18.2 mm. (range, 15.0 to 20.0 mm.) in length, and 4.7 mm. (range, 3.3 to 5.0 mm.) in width. The corn earworm pupae thus averaged 3.4 mm. longer and 1.3 mm. wider than those of the tobacco budworm. Inasmuch as these measurements were taken on laboratory-reared insects, it seems reasonable to assume that under more natural conditions the variation in length and width within a species would be greater, thereby lessening the diagnostic value of these characters.

KEY TO THE PUPAE OF *H. zea* AND *H. virescens*

Abdominal spiracles relatively large, narrow dimension of those on segment 3 equal to about one-seventh length of segment (fig. 2); maxillary palpi usually visible (fig. 4) ..... **zea**

Abdominal spiracles relatively small, narrow dimension of those on segment 3 equal to about one-fifteenth length of segment (fig. 1); maxillary palpi not visible (fig. 3) ..... **virescens**

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The Cluster Fly, *Pollenia rudis* (Diptera: Calliphoridae)[1]

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Cluster flies (*Pollenia rudis* (Fabricius)) become a serious problem and a major pest in rural homes, churches, and other structures in the autumn when they enter buildings to hibernate, and again in the spring when they seek ways to escape to the outside. Heavily infested buildings may contain several thousands of these flies. Sometimes a few thousand of them aggregate and form a "cluster," whence their popular name. While they are not filth flies, they are severe nuisances when they gain entrance and fly in huge numbers about living quarters.

Study of the life history of this fly is complicated by the fact that the larva feeds only on specific earthworms. During the first two stadia it feeds as an internal parasite, and during the last one it attacks as a predator from the outside. The dependency of the cluster fly upon earthworms (*Helodrilus chloroticus* (Savigny)) was first described by Keilin (1911) in France. In the light of subsequent data, however, his account of the feeding behavior of the larvae is to be questioned. McDaniel (1927) reported cluster flies as breeding similarly to house flies. DeCoursey (1927a), a prodigious worker in this area, failed in attempts to feed the larvae on foods other than living earthworms (*H. chloroticus* and *H. roseus* (Savigny)); he used manure loam soils, grass sod, decaying meat, and dead earthworms. Tao (1927) reported his inability to rear larvae of *Pollenia rudis* on any common blow fly media. In the few recorded cases of successful rearing of the fly (Garrison 1924, DeCoursey 1927b), only a small number were produced, the stages of larval development were not observed, and the process was not easily controlled. Other procedures involved using boxes of moist soil or flower pots which contained the earthworms, placing flies in cages over the soil, and removing the adult flies following oviposition. Our attempts to rear cluster flies by these procedures failed.

The aim of this investigation was to develop a technique for rearing *Pollenia rudis* in such a way that an appreciable number of flies might be produced and development could be observed, so that more information on the ecology of the species might become available.

PROCEDURES AND RESULTS

Adult flies were net-captured most easily and in greatest numbers between sunrise (about 6 a.m.) and 7:30 a.m., on the east side of white farm buildings. At that time of day, cluster flies made up 98 percent of the insects resting on the buildings. Collections were best on warm mornings, sunny or cloudy, and poorest on those that were rainy, windy, or cool (below 60° F.).

The flies collected were maintained in 1-gallon, wide-mouth glass jars with screened lids. In each such cage a petri dish was placed which contained a small paper cup, 1 inch in diameter and 1 inch deep, with powdered skim milk and granulated sugar (1:1), and a 4-dram shell vial, full of water and stoppered with a cellucotton plug. Within 2 to 4 days after capture of the adults, cluster fly eggs were found on the bottom of the jar, in the petri dish, and often in the crevices of the cellucotton. The eggs collected were transferred to a petri dish containing two thoroughly dampened filter papers, where they hatched in 2 to 3 days at about 80° F. The newly emerged larvae were removed with a camel's-hair brush for use.

Attempts have been made to feed larvae of *P. rudis* the following species of earthworms: *Lumbricus terrestris* Linnaeus, *L. rubellus* Hoffmeister, *Octolasium lacteum* Orley, *Helodrilus foetidus* (Savigny), *H. roseus* (Savigny), *H. caliginosus* (Savigny), and *Diplocardia communis* Garman. If these worms were wounded the larvae would feed, as was evidenced by the material in the gut. However, the only species that *Pollenia rudis* penetrated by itself was *H. roseus*. Only one specimen of *Helodrilus chloroticus* was ever collected in the vicinity of Ithaca, so this species was not included in the tests. *H. roseus* was found in damp to wet soil, and was most abundant along stream banks. The worms were maintained at 65° F. in the moist soil from which they were collected.

Three physical environments were found satisfactory for maintaining *Pollenia rudis* and earthworms together. In the first, the sides and bottom of shell vials, 0.4 inch in diameter by 1 inch high, were lined with filter paper. This absorbed the slime which could trap young fly larvae. One *H. roseus* was placed in each vial, along with some rotting maple leaves. Because larval mortality was found to be high, about eight cluster fly larvae were placed in each vial, and a larger vial was then slipped over the smaller one to form a cage. This small cage was forced into a ½-inch layer of damp sand present inside a 1-gallon, wide-mouth glass jar. The entire arrangement was then placed in a constant-temperature cabinet set at 65° F. The damp sand provided a microclimate of 100 percent relative humidity.

In the second arrangement (suggested by E. F. Menhinnick), a block of plaster was cast which was 3 inches wide, 9 inches long, and 1 inch thick. Before it hardened, 12 depressions, each ½ inch in diameter and ½ inch deep, were made in the plaster. After hardening, the block was soaked in water to dampen it thoroughly. One *H. roseus* was placed in a depression, along with rotting leaves, and again about eight *P. rudis* larvae were introduced per worm. The entire block was covered with a glass plate to confine the worms, and was then kept in the dark at a temperature of 65° F.

Though cluster fly larvae were reared successfully by both of these techniques, adequate observation was not possible unless the equipment was disturbed. Therefore a third, more satisfactory, type of environmental setup was devised.

Shell vials, 0.6 inch in diameter by 1.8 inches high, were filled one-third full of a stiff agar (5 grams in 100 ml. of water). This provided a firm, moist shelter for the earthworm and also permitted a high degree of visibility. Just prior to its insertion in a vial, each earthworm was allowed to crawl across dry filter paper, which removed the slime secreted during handling. A single worm was placed in each vial, the rest of the space was loosely packed with crumbled, well-rotted leaves, and the vial was then lightly stoppered with a cork. This seemed to permit passage of enough air to prevent harm to the earthworm and the fly larvae. Before the larvae were introduced, the vials were kept at 65° F. for 2 days to let the earthworms become accustomed to their new habitat. As before, about eight larvae were placed on each worm, and the vials were then left undisturbed for 4 days before examination. In every case of successful penetration the larva entered the worm anywhere behind the clitellum. Its posterior segment could be observed through the vial.

To increase the number of penetrations of *P. rudis* larvae into the earthworm, a procedure was improvised for cutting a small opening in the dorsal surface of the latter. A small cut was made about half-way across the body wall, about 25 segments in front of the anal segment, and the wound was dried with filter paper. A larva of *P. rudis*, head-end-first, was placed in the open wound, the edges of the wound were allowed to heal about the larva, and the worm was then placed in a shell vial-agar cage. This procedure increased the number of larvae that successfully established themselves in the earthworms.

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The larval stage of *P. rudis* lasted about 21 days at 65° F. Larvae could reach maturity on a single *H. roseus*, but would feed on as many as three earthworms if they were provided; in the latter instances, large-sized pupae resulted. The pupal period was about 7 days at 80° F. and 100 percent relative humidity.

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BOOK REVIEW

THE CONTROL OF GROWTH AND FORM: A STUDY OF THE EPIDERMAL CELL IN AN INSECT, by V. B. WIGGLESWORTH. Cornell Univ. Press, Ithaca, N. Y. 140 pp., 47 figs., 1959. Price $3.00.

Prof. Wigglesworth's authorship is a guarantee of fine craftsmanship in both experimental science and literary endeavor and this small volume, which comprises the six Messenger Lectures given at Cornell University in 1958, is no exception. The work deals very largely with the author's original researches on the triatomid, *Rhodnius prolixus*. This restricted subject matter is clearly justified as the author points out, in that the study of growth embraces a vast section of biology which presently can be understood only by a detailed consideration of a limited portion of the subject, and further that the clearest understanding of cellular functions is obtained by study of highly specialized cellular systems rather than the almost hopeless complexity of such a cell as the amoeba. The six chapters: the epidermal cell, the capacity for differentiation, hormones and the control of growth, hormones and the control of form, polymorphism, and integration of growth—form a beautifully balanced account of the current state of knowledge of insect growth. In these the reader is gently and painlessly led through the biochemical complexities of cuticle formation, and the role of hormones in molting and metamorphosis, to a satisfying picture of the integrated and developing organism as a sort of giant molecule existing as a chemical continuum. The book is beautifully illustrated with both photomicrographs and line drawings, including many which demonstrate the author's masterful experimental techniques. It is a pleasure to recommend this volume to all experimental biologists, for reading it is not only intellectually stimulating but also produces an almost irresistable desire to hasten to the laboratory and join in the fascinating investigations on the processes of growth and differentiation.

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ERRATUM

In volume 53, number 3, page 356, column 2, lower middle, concerning distribution of *Cacoxenus inquilinus* Hendel: "Known only from Australia", should read "Known only from Austria."

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VOLUME 1, NUMBER 2, pp. 33–68 appeared February 17, 1960 and contains a "Phylogenetic-Systematic Study of Larval Oedemeridae (Coleoptera)" by Jerome G. Rozen, Jr.

VOLUME 1, NUMBER 3, is now in preparation. It will contain the following: "A Revision of the Genus *Chlaenius* Bonelli (Coleoptera, Carabidae) in North America," by Ross T. Bell. "Swarming and Mating in Mosquitoes," by Erik Tetens Nielsen and James S. Haeger.

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